ABSTRACT OF THESIS

Name of Candidate M.M. IKEME B.Sc.(Hons.Zool.),Dip.Biol.

Address 7A Relugas Road, Edinburgh, 9.

Degree Doctor of Philosophy *Date* November 1964

Title of Thesis "Studies on Ascaridia galli (Schrank, 1788), (Nematoda: Ascaroidea) in domestic fowls, with particular reference to the dynamics of parasite populations in relation to continuing challenge of the host."

Studies were carried out on the development of Ascaridia galli infections of poultry maintained at different levels of nutrition and experimentally infected by daily ingestion of varying numbers of infective eggs.

The following results were obtained:

1. At low infective levels (10 eggs per day) a substantial development to the mature fecund adult stage occurs without evident delay. At high infective levels (1000 eggs per day) metamorphosis is retarded and larval growth is arrested in the third instar. Inhibited larval populations persist for at least 19 weeks and it is suggested that they derive entirely from those which enter early in the infection history. Sporadic resumption of development with the subsequent appearance of adults occurs late in the infection. It is held that both inhibition and resumption of development are controlled by the internal environment of the host and are manifestations of host resistance. This host resistance is relatively non-operative against low challenge levels and is severely impaired in conditions of malnutrition even against high challenge levels.

2. Resistance, as manifest in inhibition of metamorphosis increases with increasing age of the host. The phenomenon is most evident when comparison is made between younger and older birds during a first experience of infection.

3. Antibody to helminth antigen is identifiable by a haemagglutination technique. Antibody develops in response to infestation following high challenge but is barely measurable when the infestation results from low challenge. In severe malnutrition the antibody mechanism fails.

4. Inhibition of the antibody mechanism by X-irradiation and 6-thioguanine administration was demonstrated using bovine serum and helminth infestation. Infected birds with an inhibited antibody mechanism showed no significant difference in their resistance capabilities from normal birds. Resistance is, therefore, independent of antibody production.

The significance of the findings is discussed.

Use other side if necessary.

STUDIES ON *ASCARIDIA GALLI* (SCHRANK, 1788), (NEMATODA: ASCAROIDEA) IN DOMESTIC FOWLS, WITH PARTICULAR REFERENCE TO THE DYNAMICS OF PARASITE POPULATIONS IN RELATION TO CONTINUING CHALLENGE OF THE HOST

by

M.M. Ikeme B.Sc. (Lond.) Dip. Biol. (Edin.)

Thesis presented for the Degree of Doctor of Philosophy of the University of Edinburgh in the Faculty of Science, 1964.

CONTENTS

INTRODUCTION

The most frequently occurring and numerically abundant parasitic nematode in the domestic fowl is generally known by the name Ascaridia galli (Schrank, 1788). It is found normally in the small intestine. This worm has received considerable attention from poultry workers and parasitologists and is the subject of a quite voluminous literature. This is due to the reason that the worm has a world-wide distribution, and its large size makes it conspicuous at autopsy. For the same reasons, its pathogenicity to the host may have tended to be exaggerated in the past; nevertheless, it is widely held to be of serious economic importance. Its large size, moreover, makes Ascaridia galli a convenient subject for handling, and this together with the ease with which its host can be kept makes it an attractive subject for experimental work.

Taxonomy

The name Ascaridia was introduced by Dujardin in 1845, for a subgeneric entity of Ascaris to include the avian parasites in which the uterine branches were opposed, the type species being the psittacine ascarid Ascaris truncata (Zeder, 1803), (= A. hermaphrodita, Frohlich, 1789). Ascaridia was raised by Railliet and Henry (1912) to full generic status and was defined by

them in 1914 as a genus of parasites mainly of the small intestine of birds (although including one species seen in reptiles and fish) with the following characters: "Mouth with three lips, oesophagus without a bulb, often with lateral membranes. Caudal alae of males feebly developed. Pre-anal sucker rounded with chitinous ring; papillae relatively large. Spicules equal or sub-equal without an accessory piece. Vulva in females towards the middle portion of the body, uteri opposed, and eggs thick shelled." Although the classification of Railliet and Henry (1914) groups Ascaridia galli and Heterakis spp. under the family Heterakidae, this has long been remedied, for the present classification of Yorke and Mapleston (1926) classes this nematode nearer to Ascaris, and separates it from the Heterakidae by the absence of an oesophageal bulb. It is placed in a separate sub-family Ascaridiinae, Travassos (1919), in which group the males possess a pre-cloacal sucker with a chitinous rim.

The taxonomy of this parasite has in the past aroused a great deal of controversy. Railliet and Henry (1914) had listed thirty-one avian species within the genus Ascaridia. Of these, five were from the domestic fowl, namely, lineata (Schneider, 1866), brasiliensis (Magalhaes, 1892), compressa (Schneider, 1866), granulosa (Linstow, 1906) and perspicillum (Rudolphi, 1803) - a species from the intestine of turkeys and described by him as Ascaris perspicillum. Schwartz (1925) was of the

opinion that the commonest species in the U.S.A. was A. lineata since most of the material from the U.S.A. agreed closely with the original description based upon Brazilian specimens. Regarding the specimens described as A. perspicillum, he considered that the range of variation in the arrangement and location of the caudal papillae of the male was such as to suggest that several species were being confused under one name or alternatively the range within A. perspicillum was greater than the differences between it and A. lineata thus suggesting that A. perspicillum and A. lineata together represented a single variable species in America. Schwartz followed the second of these views and regarded all accounts of American Ascaridia species in poultry as probably referring to A. lineata. This was later supported by Ackert (1931) after examining a considerable amount of material from England besides specimens from hosts in the United States, Uganda, Tangier, Gold Coast (Ghana), India and Germany.

In 1923, Freeborn reintroduced the name Ascaridia galli (Schrank, 1788) for Ascaridia perspicillum, without characterising the species. This was later followed by Cram (1927) who included Ascaridia perspicillum in the synonymy of Ascaridia galli. She separated this from A. lineata by the supposed presence or absence of the lateral alae on the body, the presence of a pair of ventral papillae in the posterior part of

the tail of A. lineata and the difference in the sizes of the pre-anal sucker, and spicules, but failed to substantiate this by references to any supporting authority.

The first person to put forward the view that the names Ascaridia perspicillum, A. lineata and A. galli were synonymous was Baylis (1932). After a critical assessment of the supposed morphological differences existing between the above mentioned species from studies of the various type-specimens, he concluded that the common large round-worm of domestic fowl comprised a single species only, and he felt that it was legitimate to retain the name Ascaridia galli (Schrank, 1788). This conclusion was, however, unacceptable to Ackert (1931) who for some years continued to retain the name Ascaridia lineata as having preference over Ascaridia galli, his reason being that "while the name Ascaris galli was given by Schrank in 1788, he did not describe the parasite, but based the species on Goeze's (1728) composite species Ascaris teres, a nematode Goeze had found in dogs, cats, chickens and raptores." The view of Baylis was later supported by Frenzen (1955) who stated that by the law of priority Ascaridia galli was the valid name for this nematode.

Morphology and Life-history

The oral opening of the adult worm is surrounded by three prominent lips, one situated dorsally and two situated on the sub-ventral aspects of the body. The distal margin of each is further subdivided into three lobes. The dorsal lip bears two papillae and the sub-ventral ones bear one each. The fully grown males are much smaller than the females in size, and measure 50 to 76 mm in length, whilst the females measure between 72 to 116 mm. Other general characteristics of this worm include the absence of an oesophageal bulb, the presence in the male of small alae, a thick circular pre-anal sucker, a pair of sub-equal spicules and ten pairs of tail papillae of various sizes arranged diagnostically in four groups: three pre-anal, one anal, three post-anal, and three sub-terminal pairs, the last group being the smallest.

In the females the vulva is situated slightly anterior to the middle. The egg is oval with a smooth, thick shell covered by an outer thin, irregular, albuminous coat and is laid in a single cell stage, fertilized or unfertilized. The fertilized egg measures between 73 to 93 u by 46 to 57 u, and has a clear central-spot, the nucleus. The unfertilized egg can easily be recognized by its large size and the absence of a central nucleus, the whole shell being

filled by a heterogenous mass of protoplasm fat and yolk. This type of egg never starts to cleave, and soon dies.

The first attempt to work out the life-history of this worm was by Scott (1913) who showed it could be transmitted by earthworms but was not certain whether the earthworm acted as an intermediate host or transport host. The present knowledge of the life-history, however, is mainly from the works of Ackert (1923 & 1931) and Roberts (1937). The eggs are passed out uncleaved, and under suitable conditions of temperature and moisture develop to the infective stage after the developed larva has completed one moult in about 16 days. Further development of the second larval stage is arrested until the egg is swallowed by the host. Hatching occurs mainly in the duodenal region of the intestine, and the larvae may penetrate the duodenal mucosa where they remain with their heads buried between the villi or the crypts for about seven days after which they re-enter the lumen of the intestine. If, however, hatched larvae are swallowed, they are unable to establish in the host. On re-entering the intestinal lumen the larvae undergo three further moults to become young adults. Penetration of the liver, lungs and other organs of the body by larval migration is extremely rare and such migration is abnormal.

Distribution and Economic Importance

The problem created by this worm has been recognised by the poultry industry for a very long time because of its world-wide occurrence in birds of all ages of both domestic and guinea-fowls. One of the most outstanding names associated with the studies of this worm is that of Ackert. As early as 1923, he had worked out a detailed life-history and had begun a study of its bionomics. Since then other workers, like Roberts, Graham and Porter, were to produce much of the information on which present knowledge of the biology of this parasite is based.

As early as 1896, Cobb held that when these nematodes occurred in numbers much above the average carried in nature by the host, they would cause serious results, especially when young chickens were infected. Bradshaw (1907) supported this view and classed the worms on an equal basis with fowl cholera regarding their distribution, destructiveness and difficulty to diagnose. Ackert and Herrick (1928) and Staffseth and Thompson (1932) reported sluggishness, loss of appetite, ruffled feathers, drooping wings, loss of blood and weight, and retarded muscular and osteological development in infected young chickens. These effects were attributed by Ackert et al. (1928) to injury caused to the intestinal wall by the parasites with a resultant

loss of blood and probable secondary bacterial infection. Clapham (1937), from the post-mortem of a one year old guinea-fowl, reported similar symptoms, including diarrhoea, severe enteritis and inflammation especially in the duodenum and the first few inches of the ileum. The gut contents were very heavily blood-stained and consisted largely of mucus and thousands of Ascarid larvae. Although some of these were free in the gut or entangled in the mucus, the vast majority had burrowed into the mucosa causing haemorrhage and the destruction of Brunner's glands. Similar pathological findings by Roberts (1937) supported this view.

There are many other available works, reporting direct pathological changes on poultry infected with Ascaridia galli. They include those of Herrick (1926), Ackert (1931), Ackert et al. (1946) and more recently those of Sadun (1949 & 1950), Tsvetaeva (1954) and Kadziolka (1960). They are all agreed that infection often involves direct pathological effects like loss of weight and stunted growth, mucoid diarrhoea and haemorrhages in the intestinal epithelium often leading to extreme hyperplastic changes in the intestinal mucosae of the infected birds.

Apart from direct pathological changes due to infection, some work has also been done showing indirect effects. Shikhobalova and Kustova (1950) and Pande and Krishnamurty (1959) reported Vitamin A reduction in the

liver of chicks during infection. Sadun et al. (1950) reported that worms from chickens receiving large amounts of fresh liver extracts were significantly longer than those from control birds fed on dried liver extracts. He inferred that these worms required Vitamin B for their growth. This was first shown by Ackert et al. (1927). Sadun et al. inferred that a heavy infection of birds may be manifested by deficiency symptoms. Ackert and Gaafar (1949) and Gaafar et al. (1953) also recorded data which suggested that these nematodes require phosphorus and calcium for their normal development. Ackert and Whitlock (1935) and Ackert (1940) ascribed the food of the adult worms to the host ingesta.

From these reports it is apparent that the indirect pathological effects of this worm include depletion of Vitamin A stored by the host, competition with the host for Vitamin B, phosphorus, calcium and host ingesta, hence the parasites can produce deficiency symptoms in infected chickens.

From the above brief resume one can appreciate the potential importance of this parasite in relation to the economy especially of large-scale poultry production. In most tropical countries, a great part of the large-scale poultry production is of the open range system. The temperature and humidity are high, and these are conducive to rapid development of voided eggs.

Ackert (1931) reported that eggs could reach the infective stage in 16 days at 30°C, and in 10 days at 33°C; Roberts (1935) reported that the infective stage could be reached in 7 days at 33°C, and Reid (1960) obtained infective eggs in 5 days at 34°C. All these conditions of climate can be reached in the tropics, and outbreaks of Ascaridiasis may very easily be expected to reach epidemiological levels. Even in cold climatic conditions, under deep litter conditions of rearing poultry, rapid development of eggs does occur (Koutz, 1953).

An essential element in the understanding of the population dynamics of this parasite is the resistance that occurs or can develop in the host.

Immunity and Resistance

Several accounts have been given of immunity and resistance, although not all have been substantiated fully by experimental data. It has been held, however, that immunity and resistance to Ascaridia galli infections of poultry are related both to age and to previous experience (acquired immunity).

Age

Herrick (1926) was the first to report the phenomenon of age-resistance in chickens when he demonstrated that the chance of survival and growth of A. galli in poultry was inversely proportional to the age of the host. He infected chickens of ages varying from 5 to 240 days and compared the mean length of larvae after a duration of infection of 10 days. From the inverse relation between larval size and host age, he concluded that as chickens got older, they became more resistant to A. galli. The peak of resistance was found to be at 103 days. This finding was later supported by Ackert and Herrick (1928) who reported that birds of four months old were much more resistant to nematodes than chickens of one month old. Later Ackert et al. (1935) reported that age resistance increased to up to 93 days. Roberts (1937) agreed with this view. Ackert et al. (1938 & 1939) noticed an increase in the number of goblet cells with age in chickens. He

reported that the highest goblet cell counts occurred in the chickens aged 124 days. He correlated this with the age resistance reported in previous works and felt that part of the mechanism of age resistance was through the development of intestinal mucus. He cultured young Ascarids in media with added mucus and reported the occurrence of growth-inhibiting factors in this mucus. Its inhibitory effectiveness depended on the age of the chicken from which the intestinal mucus was taken. This has since been supported by Frick and Ackert (1947 & 1948) who reported that the effect was nutritional and temporary as the growth of the worms was resumed with removal of the mucus. This was not an antibody as it could not be destroyed by autoclaving and does not depend upon prior immunization of the host. This has so far continued to be held as the explanation of age resistance to Ascaridia galli in chickens.

Acquired Resistance

Evidence of acquired resistance was also first reported by Herrick (1926) who stated that there was a close relationship between the numbers of worms harboured and their size if chickens of the same age group were given a single feeding of infective eggs. Ackert et al. (1931) also showed that the establishment of worms was inversely proportional to the number of infective eggs fed. These results were considered to be due to a serological factor operating during the period when the

larvae attacked the intestinal wall. Graham et al. (1932) infected 84 chickens at 5 weeks of age, and gave them a challenge infection at the age of ten weeks. At autopsy three weeks later, they found that the lengths and numbers of the worms surviving from the second infection were indicative of a slight immunity. They had used the size of the worms as the criterion for determining the infection they came from. At a second trial, worms from the first infection were eliminated with carbon tetrachloride before the second or challenge dose and counts and measurements done as before. This result was also indicative of a slight immunity although only in one case was the difference in worm lengths statistically significant. They suggested that this immunity was due to the formation of antibody when the larval worms invaded the intestinal wall. This opinion was supported by Roberts (1937) and Sadun (1947, 1948 & 1949) who stated that a previous single or repeated infection made the chicken strongly resistant to re-infection.

The presumed antibody, however, could not be demonstrated serologically by the precipitin test or by other tests although Sadun (1947 & 1949) was able to demonstrate in vitro precipitates around the natural openings of larvae immersed in the serum of infected birds. Sadun's work also indicated that the amount of antibody produced to a given size of infection appeared to

increase with the age of the chicken. Kadziolka (1960) reported failure to identify serological reactions in infected birds, although she omitted to describe her techniques.

Several workers have tried to transfer resistance passively by serum injections but with conflicting results. Herrick (1926) claimed that serum from a 123 day old worm-free cockerel had vermicidal properties. He injected this into a 22 day old chick with a 10 day old infection and at autopsy 15 hours later noticed partially digested worms and believed that lack of living worms was due to the injected serum. Serum from the same cockerel was also given to three other chickens in doses of 1 ml, 2 ml and 3 ml respectively. On subsequent examination the chick with 3 ml of serum was free of worms while the controls which had received no serum treatments had over 2,000 larvae. A third trial gave strong evidence of a strong protective action against symptoms of parasitism although the growth rate of the worms was unaffected. The results were not very clear cut so Herrick concluded that such sera made chickens more resistant to the effect of the worms than to their establishment. Sadun (1947 & 1949) was able to transfer a measure of immunity passively by using sera from infected chickens. He concluded that immunity was partly serological.

Egerton et al. (1955), however, found that chickens

receiving immune serum harboured more worms than the control chickens although the net weight gains of the birds were greater in the former. The immune serum was obtained from 5 week old chickens given a single dose of 100 infective eggs at two weeks old and bled three weeks later. This was injected intraperitoneally at the rate of 2 ml per test bird. Test birds were killed at five weeks of age, i.e. three weeks after infection. The criteria for determining resistance were number and lengths of larvae obtained after killing. Eisenbrandt and Ackert (1940) were also unable to produce an immunity to Ascaridia galli in white leghorns following intracardial injections of specific nematode extracts. Only a third of the chickens so treated showed any signs of protection but the amount of extract injected bore no relation to the degree of resistance which followed.

Although a large number of studies have been carried out on the biology of this parasite, it is rather surprising that they nearly all refer to short-term experiments based on single infections. Very little information is available on the effect of repeated infection of this parasite to poultry.

Roberts (1937) worked on repeated infection using three groups each of five birds. He infected them, respectively, with 100 eggs per day, from the age of 30 days, 300 eggs per day, from the age of 58 days, and 500 eggs per day from the age of 79 days. The doses were

increased respectively to 300 eggs per day from the age of 85 days, 1000 eggs per day from the age of 118 days, and 1000 eggs per day from the age of 119 days. He noticed that the infections affected body weights and induced serious conditions of Ascaridiasis comparable to conditions obtained in single infections. Although recovery occurred in all groups the health again broke down when the dosages were increased.

Sadun (1948) did not carry out a daily repeated infection like Roberts (1937). He used three chickens: at 5 days old they received 7000 eggs each, 23 days later each was given 40,000 eggs, a further 22 days later they received 1250 eggs each and 18 days after this, the third infection was given when each bird received a test dose of 8000 eggs. The birds were killed 14 days later and worm counts were made. The worm counts of the three chickens were: 0, 1 and 2. From their lengths, varying from 1.7 to 2.8 mm, Sadun concluded that they all came from the test dosage.

These two works represent the only available ones on repeated infection with Ascaridia galli.

For a better understanding of the biology of this parasite, further work ought to be done based on conditions which simulate epidemiological situations in nature. Infection of poultry in nature is normally repeated. Experiments based on repeated infections are essential for studying the dynamics of populations of

this parasite under varying nutritional conditions of poultry. From this type of experiment, a lot of ground could be covered on aspects of pathogenicity and resistance. In recent years, the poultry industry has developed very rapidly and this has been accompanied by better management conditions as well as improved nutrition. Bearing this in mind, it is desirable to re-assess the previous beliefs in pathogenicity and resistance under the light of better feeding and management conditions.

The previous work summarised above had mentioned drastic effects of this parasite in poultry. Sadun (1949) had even worked out what he reported as lethal doses to poultry of various ages. He reported that 100 eggs per gram body weight would kill chickens of three days of age, and about 220 eggs per gram body weight would kill chickens 8 to 24 days of age. Previous workers had also stressed that a resistance to a secondary infection in poultry was high. A survey of these works showed that the criteria for these beliefs were based on parasitizing numbers of birds with known numbers of eggs, and collecting and measuring at autopsy all worms available. In most cases the duration of the infection had been too short for evidence of much importance to be gathered, since the number of larvae or young adults present at this stage often bore no relation to the final population of adult worms in the host.

Herrick (1926) killed the chickens 10 days after the challenge infection while Ackert and Herrick (1928), Graham, Ackert and Jones (1932), Ackert and Porter (1935), Roberts (1937) and Sadun (1948) killed their experimental birds three weeks after challenge.

From an epidemiological point of view, the criterion for determining the presence or absence of resistance ought to be based on the number of birds, if any, harbouring a worm population for a duration of time long enough to enable these worms to reach adult egg laying stage, thus constituting a danger to other chickens. Fecundity of the worms must be considered too when possible. It is obvious that larval and young adult counts done under the time intervals adopted by most previous workers do not satisfy this condition and hence results obtained on resistance, both under age, single infection, and repeated infections, are not clear cut.

Koutz (1953), studying the effect of built up litter on parasitic ova and coccidia oocysts, reported that rearing young chickens on litter used by old hens could produce grave parasitic problems to the young chickens. From his experience in nature, old hens could harbour dangerous worm populations. Field surveys in Britain, like those of Owen (1951) and, more recently, Wakelin (1964), show birds of varying ages to harbour large worm populations. These workers failed to obtain any concrete results indicative of an immunity due either to

age or repeated infection in nature. Wakelin actually obtained data which showed that old birds harboured more worms than younger ones, and the percentage of infected individuals was higher among older birds. Wakelin attempted to explain this by suggesting that younger birds received better management. One could also argue that anthelminthic treatment of adult birds in the field tended to bring down the worm population in adult birds lower than would have been expected.

Personal experience from a survey in 1961 of poultry parasites in villages in the Plateau Province and in the Egg Production Unit of the Department of Veterinary Research at Vom, Northern Nigeria, also showed this apparent anomaly of old birds harbouring more Ascaridia galli than expected and constituting a source of infection to young chickens. It is extremely difficult to explain these results in the light of published information from experiments on age, and acquired resistance in poultry.

The present investigation was undertaken to re-examine the relationship between age, infective challenge and host resistance and to reassess the epidemiological role of resistance, and thus working out the population dynamics of Ascaridia galli infections in poultry, simulating as far as possible conditions which occur in nature. This has been carried out by feeding chickens with graded doses of 10, 100 and 1000 infective

eggs per day, under varying conditions of nutrition. From this experimental model, studies on pathogenicity were carried out, and attempts made to re-examine the phenomena of resistance and serological immunity by the use of tanned red-cell haemagglutination tests.

To facilitate presentation, it has been found convenient to divide the report of the work into five distinct sections as follows:

Section 1 deals broadly with host-parasite relationships, and is concerned with the variables of nutrition, age, and previous experience of parasitic infection, under circumstances simulating natural ones. The study is carried out under a closed system of Ascaridia galli infection without concurrent parasitic infection. The chicken provides a semi-constant ecological environment permitting study of the interaction between the host's internal environment and the parasites. This includes not only such effects of the infection on the chicken as pathogenicity, but also deals with the effects on the worms of the host's internal environment as determined mainly by studies on the population dynamics, size, fecundity, etc., under different levels of continuing challenge.

Section 2 includes detailed comparative population dynamics studies of Ascaridia galli infections under repeated daily infections of 10 and 1000 embryonated eggs respectively. This involves the comparison of

weekly counts on all stages of worms in each group for a period of 19 weeks.

Section 3 is devoted mainly to serological investigations of infected chickens.

Section 4 includes a report on blood protein studies and pathological changes.

Section 5 deals briefly with the host-parasite relationship in hosts in which the mechanism of antibody response had previously been inhibited or destroyed.

Specialised methodology is described under its appropriate section.

MATERIALS AND METHODOLOGY

Chicks

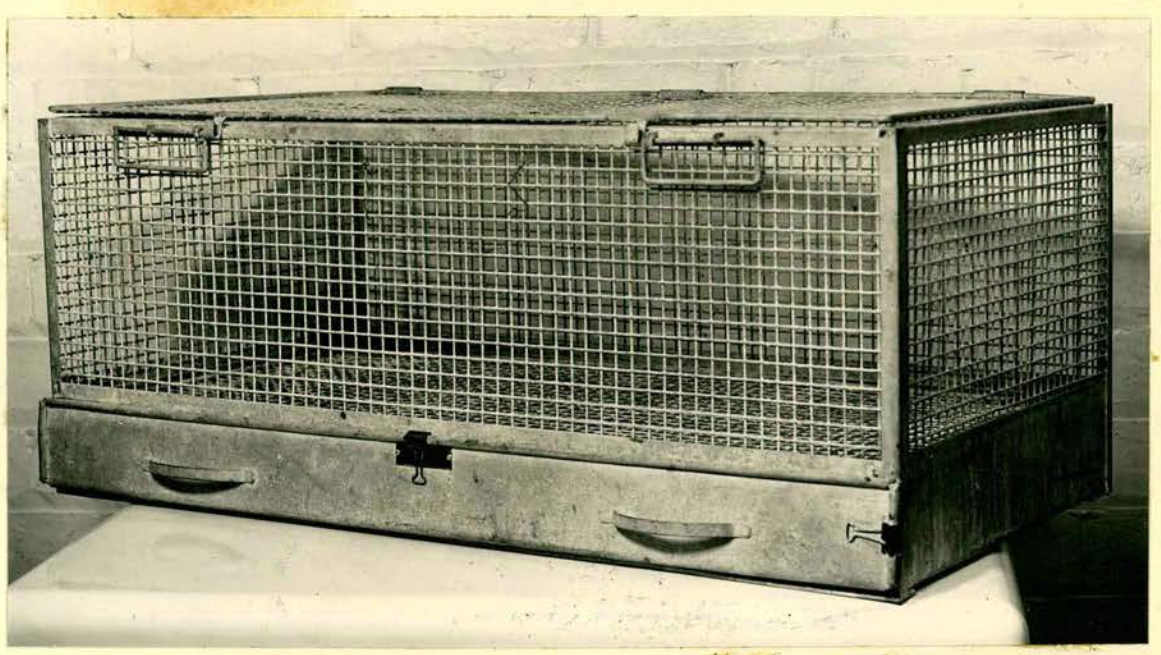
The chicks used throughout this experiment were from a non-in-bred strain of pure Brown Leghorns. Previous workers, e.g. Ackert et al. (1935), have stated that heavier breeds of chickens are more resistant than lighter breeds, and a light-breed Brown Leghorn was used here in the view that they would probably be better for comparison of pathogenic effects of the worms. The chickens were always supplied at one day old either from the Bush Station of the University of Edinburgh or from Lasswade Poultry Diseases Laboratories of the Ministry of Agriculture, Fisheries and Food. Sexing of the chickens at a day old was done using the following characteristics as a guide:

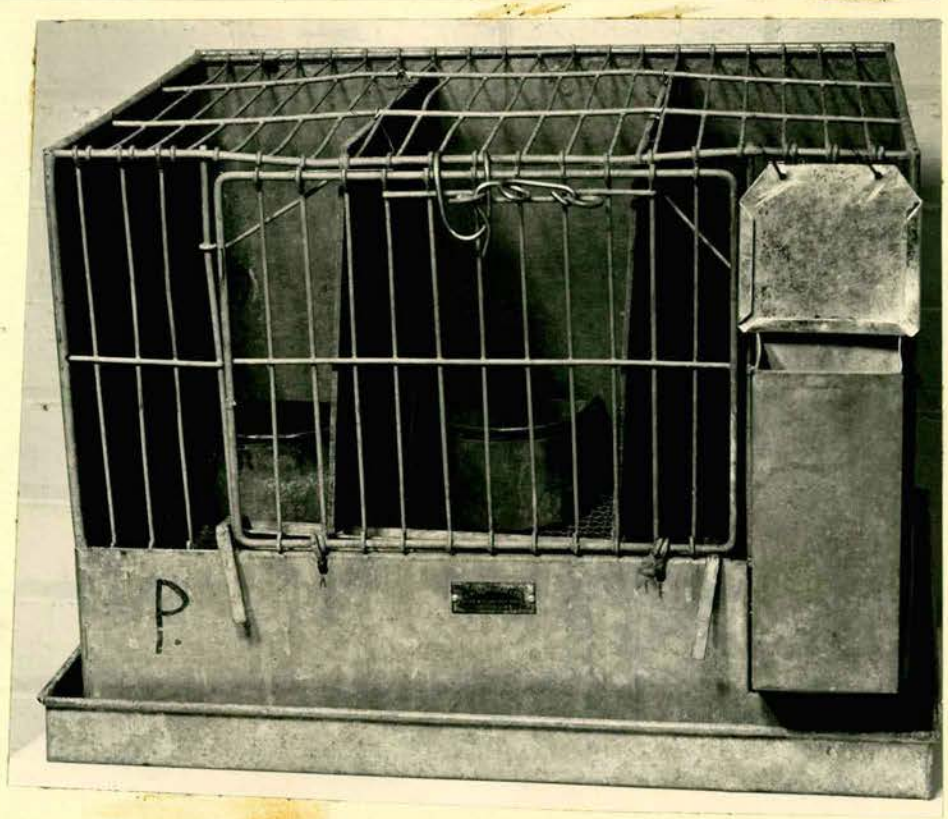
1. Down area darker in the females than in the males.
2. Line of demarcation between dark down sector and lighter portion more distinct in females than in males.
3. Face stripes darker and longer in females than in males.
4. Front border of wing medium brown in females and light brown or creamy on the males.

Only about 60 per cent of the chickens separated by this method were sexed correctly, but the use of this formula

Figure I: Rat cage used for maintaining chicks from 1 day to 2 weeks.

Figure 2: Cage for experimental birds.





was continued in the absence of any better one.

The chickens were first housed together in rat cages measuring 30 ins. by 12 ins. deep, of ½ inch square mesh, opening the whole length at the top, with 3 inch skirting at the front and a ½ inch square mesh grid floor over a 3 inch deep tray (Fig. 1). At the age of two weeks they were separated out and housed in cages measuring 20 ins. by 15 ins. by 15 ins. deep, with metal sides and back, 5 ins. deep skirting along the front, 4 ins. from either end, and hinged to the front skirting. The removable floor was of a ½ inch square mesh, and stood 2 inches above the tray (Fig. 2). The cages were all galvanised finished, and were made according to specification by E.K. Bowman Ltd., London. The second type of cage was partitioned into three sections using two cardboard sheets of suitable size, to give three equal compartments. Each contained a chick with its own feeding container of 5 ins. by 3 ins. by 3 ins. deep. A common drinking trough was fitted along the front length of the cage so that chickens from all three compartments could drink. This partitioning of cages was found to be essential during the course of the work, so as to prevent excessive pecking which occurred when chicks were housed in groups. Strict sanitation was adhered to by cleaning the cages as often as was necessary, and at the end of each experiment the room where the chickens were housed was scrubbed with hot detergent and rinsed

with a 10 per cent solution of household ammonia, and all cages and feeding vessels were washed and steamed for 15 minutes.

Worms

Gravid worms were collected from the post-mortem room of Lasswade Poultry Research Centre, and stored at room temperature until required. Trials were carried out to achieve maximum recovery of eggs and a reliable technique for completing their embryonation.

Standardization of technique for collecting ova for experiments

Several methods of getting infective ova have been described. These include culturing of the eggs within excised uteri (Riedel, 1947) or cultivating the eggs within the intact female (Riedel, 1951, & Siddiqui, 1954). In the first method, whole worms are collected and washed in 5 per cent formalin. The uteri are then pressed out, placed in fresh water and left for about 21 days for the eggs to develop. The uteri are transferred when necessary to fresh water in sterile dishes to prevent mould formation. Eggs can be liberated by transferring the uteri to a small drop of water and crushing, also when shaken in a bottle with fine sand, the egg clumps are broken up and droplets containing known numbers can readily be obtained for experimental

feeding. In the later method, whole gravid females are washed in tap-water and transferred to large, moist culture chambers to which 1 per cent formalin is added to a depth of at least 1 inch. The cultures are then incubated at about 20°C for 3 weeks. When ova are required for experiment some of the worms are ground up in pestle and mortar. The ova are then filtered through several layers of cheese-cloth, and then centrifuged down to concentrate them.

For estimation of the yield by these methods, gravid female worms were collected, washed and divided into two lots of thirty. From one lot, the eggs were incubated in the isolated uteri, while in the other they were incubated in the whole worms, in 250 ml flasks containing about 75 ml of 1 per cent formalin, and immersed in a water-bath at 25°C for 3 weeks. Aeration was maintained by small aquarium pumps, and the solutions changed every four days. At 21 days, the material from each culture was crushed with very little pressure in a mortar, and filtered into a beaker through several layers of cheese-cloth. The residue was washed and collected into a beaker, and the filtrate made up to 100 ml. This was swirled gently several times and a 0.1 ml aliquot was examined microscopically (two-thirds objective). Total egg count and total embryonated egg counts were made. Three such counts were made from each culture, and the average counts were taken. From these the percentage

of embryonated eggs in each culture was determined, and was found to be around 25 per cent of the total egg number in each culture.

Both techniques were found to have similar disadvantages in that gravid females have to be collected before incubation for infective eggs to be embarked on. The techniques involved are too tedious and unreliable as a routine method of collecting embryonated eggs for daily repeated infection. The final total yield of eggs is too low, and the percentage embryonation very poor. The latter could be due to low percentage of fertile eggs in a gravid female worm at any one time, and the gross yield, due to the technique used in liberating embryonated eggs from the uteri or from whole worms. More often than not, fungal growth is high, and using a higher concentration of formalin in the incubating solution makes the egg coats too tough. All these, coupled with the difficulty experienced in obtaining gravid females in sufficient numbers and good preservation, made these techniques very unsuitable and unreliable as a routine method of getting high yields of embryonated eggs for experiment and hence had to be abandoned.

A new method was evolved which was found to be more reliable. Infective eggs initially obtained by the above techniques were used in infecting five two week old chickens. After about 40 days faecal smears were made

to examine for eggs. When eggs were identified in the faeces, pools of freshly passed faeces from these birds were mixed into a light emulsion with plenty of tap-water in a plastic bucket. This was allowed to stand for about 30 minutes and the top layer of floating debris skimmed off. The emulsion was then washed and screened with a coarse (10 mesh per linear inch) sieve to remove coarse debris. The filtrate was then slowly poured into the cup of a chemical centrifuge set at about 5000 r.p.m. and the sediment collected in the form of a cake was mixed thoroughly in a half litre of concentrated salt solution and re-centrifuged. This time the liquid was collected and the sediment discarded. The liquid contained thousands of fertilized eggs, and these were cleaned up and further concentrated. The concentrated salt solution was diluted twenty times and allowed to stand overnight for the eggs to sediment, and the top liquid removed with a water pump. To this was added an equal volume of 1 N sodium hydroxide and left for about one hour to dissolve off the sticky albuminous coat surrounding the eggs which caused a great deal of egg loss through clumping and adherence to debris and container walls. After this treatment, the eggs were centrifuged down for 3 minutes at 3000 r.p.m., the supernatant sucked off, and the eggs resuspended in concentrated saline made up in 1 N sodium hydroxide. This further cleared up the eggs, and minimized adhesion of

the eggs and floating up of debris. The supernatant containing the eggs was collected and diluted twenty times with the embryonating fluid this time and washed. This was done twice and finally equal aliquots were distributed into 250 ml flasks and incubated in a shaker bath at 25°C for 21 days.

Efficiency of egg collection with the chemical centrifuge

Fresh faecal material was collected from infected chickens, weighed and mixed thoroughly by gloved hands. After mixing, three different samples of 3 grams were taken out and subjected to egg count by a modified McMaster method (to be described later). The bulk of the faecal material was then processed as described above and the recovered eggs made up to 50 ml. This was swirled round and immediately 0.1 ml aliquot was withdrawn on to a slide using an automatic syringe and the eggs counted. The average of five counts was taken.

The estimated efficiency of this method of egg collection is 77 per cent, i.e. 77 per cent of eggs in the faecal material could be recovered.

Fertility of eggs collected from the above method and the efficiency of various embryonating media

Formalin and potassium dichromate solutions have been used generally as media for the embryonation of nematode eggs because they are effective germicides but

do not affect the developing embryo. Siddiqui (1954), however, stated that larvae from eggs cultured in potassium dichromate solution were short lived. Trials were done to ascertain the efficiency of three embryonating media: 1 per cent formalin, 0.1 N sulphuric acid, and normal saline. Eggs isolated from faeces by the chemical centrifuge method were washed thoroughly and concentrated by centrifugation. Aliquots of 1 ml were delivered into three different centrifuge tubes, each containing 3 ml of the appropriate embryonating fluid. These were used as a final wash and they were transferred into 50 ml flasks and into one flask each was measured 10 ml of 1 per cent formalin, 0.1 N sulphuric acid or normal saline. They were covered and incubated in a shaker bath at 25°C for 21 days. After this each flask was swirled round several times in either direction, and 0.1 ml aliquots withdrawn on to three different slides. Total eggs and embryonated eggs were counted, and the average of three counts taken. The following figures were obtained:

	Formalin	0.1 N	Normal Saline
Total egg count from 0.1 ml aliquot (3)	982	1,045	-
Total embryonated egg count from 0.1 ml aliquot (3)	910	961	-
Percentage embryonation	92.67%	91.97%	-

The eggs embryonated in normal saline, but were completely covered with fungal growth and counts were impossible.

Culturing of eggs using a shaker bath was very good for periodic changes of culture media were unnecessary (a process which invariably introduced fungal growth). Sufficient aeration was obtained automatically as the shaker worked, and, furthermore, all eggs were at the same stage of development. A very high percentage of embryonation was obtained here using eggs isolated from faeces irrespective of the medium used. This could be explained by assuming that mainly fertilized eggs were passed out by gravid females.

Eggs cultured in formalin had tough coats and could not easily be broken by slight pressure under a cover-slip whilst those embryonated in 0.1 N sulphuric acid could easily be broken to liberate larvae. This toughening of egg coat was first noticed in eggs cultured in Utero in formalin. Attempts to prevent this by reducing the concentration of formalin in the culture only resulted in enhanced fungal growth.

This phenomenon could have important consequences with respect to hatchability of the eggs in the duodenum of infected chickens. If egg coats were too tough, it was felt that hatching in the chicks might prove impossible, hence from a practical viewpoint it was pertinent to emphasise that hatching of the eggs and

hence infectivity could be affected by the conditions of embryonation. Using 0.1 N sulphuric acid proved very good for culturing eggs for experiments as this did not harden the egg coat or affect the embryonation of the eggs. This supports the finding of Fairbairn (1961) who stated that formalin, like dilute acid, was an excellent inhibitor of microbial growth but reacted chemically with the shell to make it resistant to digestion by chitinase or other enzymes during hatching even though embryonation was normal. He found 0.1 N sulphuric acid to be excellent for embryonation.

Experiment to determine the hatchability of egg cultures from formalin and sulphuric acid

This pilot experiment was finally used to decide the better embryonating medium, by ascertaining the effect, if any, that hardening of the egg coats by formalin incubation had on hatchability of eggs in chickens. Two four week old chickens were used for each group (formalin and sulphuric acid incubated cultures). They were each given 1000 eggs by intubation into the crop from the appropriate culture. After 24 hours faecal material was collected from each of these chickens, mixed thoroughly, and approximately 3 grams from each were submitted to egg counts by McMaster method. The counts done were on embryonated eggs only.

Results gave egg counts of 200, and 180 eggs per gram of faeces in chickens given formalin incubated eggs, and less than 100 each in chickens given sulphuric acid incubated eggs (i.e. no eggs were seen, and sensitivity of this method is based on 100 eggs per gram of faeces or more). The results at first sight tend to suggest that hatchability of eggs embryonated in 1 per cent formalin was interfered with and much reduced, and that hatchability of eggs embryonated in sulphuric acid much enhanced. However, the possibility that embryonated but unhatched eggs from sulphuric acid cultures might have been broken up by glass beads during the emulsification of faecal material for the egg counts should not be overlooked. The investigation was not followed up but subsequent embryonation of eggs for this work was done in 0.1 N sulphuric acid as it was well able in most cases to prevent fungal growth in the cultures.

Infection

Embryonated eggs obtained by the above method can be kept in 0.1 N sulphuric acid for several months at room temperature without harmful effect on the larvae, and eggs can be used when required. However, eggs used for these experiments were never more than one month old. According to Todd et al. (1950) the peak of infectivity of embryonated eggs is reached at 21 days incubation, after which infectivity falls with storage. This was

supported by Elliot (1954) who correlated this fall in infectivity with fall in fat content of the larvae.

To determine the percentage of embryonated eggs living at the time of feeding, a drop of culture on a slide was placed on a Maclaren's controlled hot stage for microscopes, set for around 37°C, and the percentage of eggs showing motile larvae was ascertained. Motility was taken as the criterion for deciding infectivity.

Before infecting chickens, eggs from the sulphuric acid cultures were washed to remove the acid and then suspended in a 1.25 molar solution of cane sugar after the method of Hansen et al. (1954). Many previous workers had used tap-water as the suspending medium for preparing infective doses (e.g. Ackert, 1931). Hansen et al. (1954), however, found that the use of tap-water for egg suspension caused undesirable rapid settling and clumping of eggs as sampling progressed, in spite of constant agitation. Hence this was often reflected in experimental infections by greater worm burdens in chickens receiving later doses. The use of 1.25 molar sucrose solution, which has approximately the same specific gravity as the eggs, as suspending medium for infection gave a uniformly dispersed suspension of eggs which did not vary appreciably during sampling provided that adequate swirling of the bottle was done. This medium did not harm embryonated eggs since larvae were still found to be alive in eggs suspended at room

temperature in this medium for four weeks, although infertile eggs had collapsed.

Infection of chickens was carried out by squirting the desired volume of egg suspension into the crop through a three and a half inch long needle with a blob of solder at the tip. Studies were carried out on the effect of 1.25 molar sucrose solution, 0.1 N sulphuric acid and 1 per cent formalin on larvae hatched out mechanically with a sodium hypochlorite solution (milton). It was noticed that infective larvae all died coiled after 3 minutes in 1 per cent formalin, after 15 minutes in 1.25 molar sucrose and badly shrunk. In 0.1 N sulphuric acid they survived for up to 7 hours when death in elongation occurred. Several larvae were still alive in normal saline after 48 hours. These results question the advisability of using sucrose solution of this strength as suspension medium for eggs for infection. Chickens infected in that way, however, showed no noticeable adverse effects on larval establishment in comparison with those fed eggs suspended in water, as several adult worms could be recovered later. Still the question remained as to the percentage of the hatched out larvae which were able to establish before they died due to the effect of the osmotic pressure of the suspending medium.

To counteract and to minimize the possibility of this effect, the egg suspensions for experiments were

made up so that the desired number of embryonated eggs would be contained in about 0.1 ml to 0.2 ml of sugar solution so that the osmotic effect of this, if any, would be very low and also the same in all birds infected and also the controls irrespective of the dosage rate. As long as embryonated eggs were washed before suspending in 1.25 molar sucrose, the effect of the embryonating liquid on hatched out larvae was nil.

Feed

Food material used throughout this experiment consisted of chick crumbs of about 19 per cent crude protein, 3 per cent oil and 5 per cent fibre; Grower's mash of 15 per cent crude protein, 3 per cent oil and 6 per cent fibre; and maize meal of approximately 10 per cent protein. These were supplied from Scott's Hyline Feeds Ltd., Colinton, and guaranteed free from anthelmintic mixtures and coccidiostats. Day old chicks were normally started on chick crumbs and then placed on the experimental ration at the age of two weeks.

Three grades of diet were used for the experiment, the differences between them being mainly in their protein content. The highest protein percentage in the experimental diet consisted of 15 per cent, obtainable in Grower's mash. The lowest protein diet consisted of 10 per cent protein, obtainable in pure maize meal supplemented with vitamins and minerals as described.

The intermediate protein diet consisted of approximately 12.5 per cent protein, obtained by a one to one mixture of Grower's mash (15 per cent protein) and maize meal (10 per cent protein).

The maize meal vitamin and trace element (iron, cobalt, manganese, iodine, zinc and copper) deficiency were corrected by the addition of Eves' No. 9TE "Vitamin and Trace-Elemented Supplements" at the rate of 4 oz. per ½ cwt. The vitamins and trace elements in this supplement were dispersed on a toasted wheat base producing free-flowing supplements for easy dispersion in animal feeds. The protein content of the base is in the neighbourhood of 10 per cent and the natural mineral content is also very low, just as in wheat, hence the protein and natural mineral contribution to the food mixture at the proportion of mixing is more or less nil. The mixture and the relevant information were supplied by V.W. Eves and Co. Ltd. of Ilford, Essex.

Calcium and phosphorus levels in the maize meal were made up to the level in Grower's mash by adding appropriate amounts of calcium carbonate and calcium hydrogen phosphate according to specifications from the National Academy of Sciences, National Research Council Publication 827, 1960: 1. Nutrient Requirements of Poultry. The specifications are as follows:

Required amount of phosphorus in feed - 0.6%

Required amount of calcium in feed - 1.0%

Amount of phosphorus in maize meal - 0.3%

Amount of phosphorus to be supplemented in maize meal - 0.3%

Amount of calcium in maize meal - 0.02% (negligible)

Amount of calcium to be supplemented in maize meal - 1.0%

It was convenient to make up 6000 grms of maize meal at a time and the weights of supplements were as follows:

Maize meal	-	6000	grms.
Calcium hydrogen phosphate	-	79.2	grms.
Calcium carbonate	-	90	grms.
Eves' 9TE	-	26.8 (i.e. ½ lb./cwt.)	grms.

SECTION 1

HOST-PARASITE RELATIONSHIP

Material and Methods

Haematological investigations

Blood for these examinations was obtained by heart puncture using needles of appropriate sizes. About 2 ml of blood were collected each time (or less when chickens were under 4 weeks) and these were run into Bijou bottles containing Wintrobe's anticoagulant. This was swirled gently till proper mixing of the blood and the anti-coagulant were obtained. Formulae of reagents are included in the appendix.

Packed cell volume percentage determinations (P.C.V.)

The above method of blood collection was used when the P.C.V. of several birds were determined. If, however, fewer birds were being studied, heparinized capillary tubes were used to suck up blood straight from a puncture of the wing vein. When oxalated blood was used, plain capillary tubes were introduced in a slanting position into the bottles. The blood normally ran up the capillary tubes without the aid of suction, to within an inch of the end. The unfilled ends were then sealed in a small Bunsen flame.

A Hawksley micro-haematocrit high-speed centrifuge

was used which had enough slots to carry 24 capillary tubes. No balancing of tubes was needed, and the centrifuge developed 12,000 X gravity giving complete cell-packing with all types of blood within five minutes.

The tubes were placed in numbered slots with the sealed ends pointing outwards and the centrifuge switched on with the automatic time switch set for five minutes. The percentage P.C.V. was read off from the micro-haematocrit reader.

Haemoglobin determination

The method of choice was Wu's alkaline haematin method modified by Clegg and King (1942). This was excellent for rapid routine determinations because of its simplicity and reproducibility. This procedure was based on diluting a small measured sample of blood with a hundred times its own volume of 0.1 N sodium hydroxide, heating in a boiling water bath for a few minutes and cooling. The intensity of the brownish-red colour produced was proportionate to the haemoglobin content of the blood. The intensity of colour was compared with an Artificial Standard developed by Gibson and Harrison (1945) and marketed by B.D.H. as the Gibson-Harrison Artificial Standard. This consisted of an aqueous solution of chromic potassium sulphate (derived by the reduction in the presence of sulphuric acid of potassium dichromate-analar) and cobalt sulphate. This

preparation was stable, and reproduced closely the absorption spectrum of blood treated with dilute alkali, over a wide range of wavelengths. The haemoglobin values of the standard had been determined by a comparison with a series of blood samples of known oxygen and iron capacity and adjusted to equal 16 grms of haemoglobin per 100 ml by iron content of blood.

Procedure

0.05 ml of blood was sucked up using a 50 cu mm haemoglobin pipette, the blood adhering to the outside of the pipette being cleaned off with tissue paper; this was introduced into 5 ml of 0.1 N sodium hydroxide solution contained in a test tube. This solution was sucked up and down three times into the pipette to wash it out. The contents were then thoroughly mixed while avoiding foaming. An equal volume of Gibson-Harrison Artificial Standard was introduced into a separate test tube, and all the tubes placed in a metal test-tube rack and immersed into a vigorously boiling water-bath so that the liquid in the tubes was just covered by the water. Heating was timed for exactly four minutes. The rack was then removed and cooled in cold water.

Photometric determination of colour density was done using an E.E.L. calorimeter which had been zeroed with 0.1 N sodium hydroxide, utilizing a Chance OGR 1 green glass filter. The haemoglobin content of the

test blood was determined by comparing it with the reading of the Standard. The blood iron capacity of the Standard (as opposed to the oxygen values) was used for the calculation.

Weight measurements, flotation and egg count techniques

Chickens were weighed with a beam balance (E.T.A. Instruments Ltd.) capable of weighing to 2110 grms. The chickens were held steady with a rubber band of appropriate strength.

Flotation method of concentrating eggs

This was a very efficient method of concentrating eggs from faeces for various purposes, especially when the detection of the smallest quantities was required, e.g. during studies of pre-patent periods at various grades of infection.

Faeces were collected from overnight droppings of chickens, mixed thoroughly, and adequate amounts placed in 4 oz wide-mouthed, glass-stoppered bottles containing some glass beads. Twenty mls of water were added to each bottle and the faeces emulsified by swirling the bottle. The mixture was then strained with a coffee sieve. The strained material was then poured into a centrifuge tube and centrifuged for 3 minutes at 2500 r.p.m. The supernatant was sucked off using a water pump. The sediment was then broken up with a

glass rod, and the centrifuge tube filled up with saturated cane-sugar solution and covered with a coverslip. The coverslip was removed after 30 minutes, mounted fluid side on a slide, and examined for eggs under the low power magnification of the microscope.

Technique for egg counts

Collection of faeces for egg counts was done as above. Approximately 3 grms of the mixed faeces were placed in a 4 oz wide-mouthed, glass-stoppered bottle containing some glass beads. The method used was that of McMaster, with a slight modification. Ascaridia galli eggs when passed out have an albuminous coat round them, and consequently are very sticky. This tends to produce a lowering of the egg-count through the eggs sticking to the walls of the bottle used in breaking down the faeces. A slight modification was introduced to correct this.

Forty-two ml of water was measured into the glass bottle containing glass beads and faeces. This was swirled round till all the faeces were broken up. Occasionally it was necessary to use warm water to hasten this. The emulsified faeces were then strained with a coffee sieve, and the strained substance transferred into a large centrifuge tube and centrifuged for three minutes at 1500 r.p.m. The supernatant was sucked off with a water pump and the sediment broken

with a glass rod. Forty-two ml of saturated salt solution was now used in washing the sediment into the glass bottle. This was again gently swirled to break up any sedimented material not already broken up, and also eggs adhering to the glass bottle were now extracted. Immediately after swirling, sufficient fluid to fill one counting chamber was withdrawn with a bulb pipette and run into a counting chamber. This operation was repeated again to fill the other counting chamber. All the eggs in the two separate chambers were counted. To obtain the number of eggs per grm of faeces the total count was divided by two and multiplied by a hundred.

Examination of birds after slaughter

At the end of each experiment, chickens were killed, opened up and examined. The intestines were removed, and regions for sectioning cut out, and the rest washed with warm water to liberate adult worms into a sieve of 50 mesh per linear inch. These were counted and measured when required.

Examination for larval worms

When larval worms were to be studied, the intestines were cut out as soon as the chickens were killed, slit open the whole length and immersed in 100 ml of digesting fluid in a 250 ml flask. The digesting fluid consisted

of a 3 per cent pepsin solution made up in 0.5 per cent hydrochloric acid. The whole was incubated overnight (with occasional swirling of the flask when possible) at 37°C. This led to the complete digestion of the intestine liberating any embedded worms, some of which could still be found to be alive. Before counts were made, the digested material was coloured with a bit of eosin. The flask was swirled round to mix the fluid thoroughly, and a 1 ml aliquot quickly withdrawn with an automatic syringe into a petri-dish with lines ruled at approximately 2 mm intervals. This was repeated twice more, and the total larval and differential counts were done using a Watson Barnet binocular microscope at 10 x eyepiece and 2.5 x objectives. Larvae of more than 2 mm in length were regarded as third stage larvae and larvae of more than 5 mm long with definite sexual differentiation were regarded as fourth stage larvae (Roberts, 1937). Larvae of less than 2 mm in length, however, were classed as second stage larvae. Total counts for each category were established by determining the average of three 1 ml counts and multiplying by one hundred.

Histological examinations

Histological examinations were carried out for the study of:

a) Pathological changes in intestinal tissues due

to infection.

b) Penetration of larval worms into the intestinal mucosa with possible host reaction.

c) Effect of adult worms on the mucous membrane especially during obstruction.

d) Effects of the presence of infection on the goblet cells.

Tissues for sectioning were cut out as soon as the chickens were killed, and washed in fixative before finally placing in the fixing solution. The fixatives used were formol-saline-sublimate and Bouin. Exposure to formol-saline-sublimate or Bouin's fixatives was continued overnight after which the tissues were transferred directly into 70 per cent alcohol without washing. Dehydration was carried out in graded ethanol, through two changes of absolute ethanol and cleared in xylene for 15 minutes. Embedding was completed after three changes in paraffin wax at 60°C (M.P. 56°C) of 30 minutes duration each. Sections were cut with a Spencer rotary microtome, set at 5 u, and stained in Delafield's haematoxylin and eosin for general staining and with Mayer's muci-carmine for mucus-secreting cells. Sections of materials fixed in formol-saline-sublimate were first treated with Lugol's iodine, washed in tap water and decolourised in 0.5 per cent sodium dithionate before staining was embarked upon. For examination of goblet cells, sections were stained for 30 minutes in muci-

carmine after prior staining with haematoxylin. Dehydration was done through graded ethanol, cleared in xylene and mounted in D.P.X.

Experiment 1

Campbell and Gardiner (personal communication) have found a statistical correlation between declension of P.C.V. and faecal egg counts in sheep experimentally infected with Haemonchus contortus. Ross and Armour (1960) have made practical use of the P.C.V. as a measure of damage sustained by cattle naturally infected with the same worm. This species, however, is a known blood-sucker. Although A. galli is not considered to be haematophagous, Sadun (1950) reported the occurrence of a mild anaemia. Other evidence of pathogenic effects of this worm include marked atrophy of the thymus gland, reduction of blood sugar levels, and excessive deposition of urates in the ureters. Ackert (1930) explained these as due to a combination of factors like injury to the intestinal mucosa and bacterial infection leading to haemorrhage and marked distension of the blood vessels of the parenchymatous organs. Blood-stained mucosae and bloody stool due to severe intestinal lesions have been shown also by Clapham (1937), Roberts (1937) and others. All these tend to indicate that even though Ascaridia galli does not suck blood at any stage of its life-history, loss of blood by the host is inevitable when larvae migrate into the mucous membrane.

Two studies on the haematology of A. galli infection have been carried out to date by Ackert (1946)

and Sadun (1950). Ackert studied changes in blood sugar and haemoglobin concentration of 24 day-old chickens with a moderate infection of 200 eggs for a duration of four weeks, the blood examination being carried out two weeks after infection and at slaughter two weeks later. Sadun, however, studied packed cell volume percentages of three groups of chickens aged nine days. One group was given a single dose of 14,000 embryonated eggs, the other a single dose of 500 embryonated eggs, whilst the third group was used as controls. Blood cell counts and packed cell volume determinations were carried out ten days after the infection in the controls as well as in the first group. Ten days later this was repeated in these groups as well as in the second group which received a single dose of 500 infective eggs.

These two works were carried out with single dosages of infective eggs. Under repeated infection, the blood loss due to larval damage to the mucosa might possibly show a cumulative effect on the packed cell volume and haemoglobin values of infected chickens. Moreover, the conventional method of determining packed cell volume percentages which requires minimal amounts of blood through utilization of capillary tubes and centrifugation at high speed as described under Materials and Methods is more reliable than the former methods involving centrifugation of blood in ordinary

centrifuges for as long as thirty minutes. Complete cell packing for P.C.V. determinations depends on higher gravitational force and not the time factor.

From the above considerations, it was thought desirable to repeat these blood studies under repeated infection at various levels in comparison with a single infection, to see whether there exists any cumulative anaemia due to repeated infection. At the same time a preliminary study was undertaken on the pre-patent periods of infection at various dosage levels.

Six groups of five chickens were used. They were reared on chick crumbs until two weeks old and then put on the experimental diet consisting of Grower's mash containing 15 per cent protein. Food and water were given ad lib.

Group 1 consisted of control chickens without any infection, but given a volume of sugar solution equal to that used in suspending infective eggs.

Group 2 received a repeated infection of 10 eggs daily.

Group 3 received a repeated infection of 100 eggs daily.

Group 4 received a repeated infection of 1000 eggs daily.

Group 5 received a single infection of 500 eggs at the beginning of the experiment.

Group 6 received a single infection of 1000 eggs at

TABLE 1A. EXPERIMENT 1.

Individual Packed Cell Volume Values at Weekly Intervals

Group	Chicken	At Infection	Week 1	2	3	4	5	6	7
1	428	24	28	29	31	30	31	32	33
	429	24	27	28	22	29	29	30	31
	430	25	28	30	33	32	32	33	34
	431	23	28	29	31	32	33	33	32
	432	25	27	29	32	31	31	32	35
2	413	24	26	27	29	30	32	31	34
	414	23	26	28	29	30	31	32	31
	415	25	25	30	31	31	33	31	32
	416	23	25	27	28	29	30	31	31
	417	23	28	30	30	30	29	31	32
3	407	24	25	31	32	32	33	32	31
	408	25	26	27	29	29	30	30	31
	409	23	24	30	30	31	31	30	32
	410	22	25	30	30	30	30	31	29
	411	25	26	27	28	29	29	31	33
4	402	25	26	29	31	32	32	30	31
	403	24	26	27	30	32	33	32	33
	404	25	27	28	30	31	31	31	33
	405	24	25	28	30	31	31	29	32
	406	23	25	26	29	30	32	30	30
6	419	25	28	29	30	29	30	32	32
	420	24	29	28	29	30	31	33	33
	421	22	27	29	28	29	30	31	32
	422	23	26	30	32	31	31	30	32
	423	26	27	28	29	32	33	32	33

TABLE 1B. EXPERIMENT 1.

Individual Haemoglobin Values at Weekly Intervals

Group	Chicken	At Infection	Week 1	2	3	4	5	6
	428	8.6	8.9	9.5	9.3	9.6	8.0	10.2
	429	7.0	8.9	8.9	9.2	9.6	12.0	10.8
1	430	6.5	8.2	9.8	10.0	10.5	10.5	9.5
	431	6.9	10.2	8.6	9.4	10.0	9.5	11.2
	432	6.3	8.9	9.2	9.4	10.2	10.5	9.8
	413	7.5	8.7	10.1	9.3	9.5	9.8	10.0
	414	6.9	8.4	10.2	8.5	9.6	10.0	10.2
2	415	6.8	9.2	10.0	9.8	9.5	8.7	8.2
	416	6.9	9.2	9.8	9.6	9.4	9.8	12.1
	417	8.3	8.4	9.4	10.3	10.1	10.8	10.4
	407	8.3	9.6	10.1	9.9	9.6	8.6	10.2
	408	6.9	8.0	8.9	8.4	8.9	10.8	12.0
3	409	6.3	8.0	10.0	9.1	9.8	9.8	8.0
	410	7.5	8.7	9.2	9.3	9.4	10.5	10.1
	411	6.4	8.1	10.0	8.5	9.1	9.5	9.8
	402	8.6	8.9	8.9	9.8	10.6	9.6	10.0
	403	6.3	9.0	9.2	10.3	8.1	8.6	9.5
4	404	7.5	8.7	9.4	7.8	9.5	9.5	12.0
	405	7.5	9.0	7.7	10.2	8.9	9.1	6.8
	406	6.9	8.6	10.1	9.9	8.6	9.4	9.6
	419	6.6	8.0	9.2	8.9	9.0	10.0	9.8
	420	7.2	9.1	10.1	10.4	10.2	10.4	11.5
6	421	6.8	8.5	8.9	9.2	9.5	9.8	9.2
	422	6.9	9.2	9.6	10.0	10.2	10.0	9.5
	423	9.0	9.4	9.8	9.6	9.8	11.0	11.4

the beginning of the experiment.

Group 7 received a single infection of 10,000 eggs at the beginning of the experiment.

The experiment was started when the chickens were two weeks old. Packed cell volume and haemoglobin determinations were carried out before the infection on Groups 1 to 4 and 6 inclusive. This was designated 'week zero' and subsequent weekly examinations were carried out on these groups. The infective dosage and haemoglobin determinations were terminated after six weeks and P.C.V. measurements after seven weeks.

Results

The individual weekly estimates of P.C.V. and haemoglobin are presented in Tables 1A and 1B respectively. The group means of these values are graphically shown in Figures 3 and 4.

Analysis of variance of the results showed no significant differences in the P.C.V. or haemoglobin values of the experimental birds throughout the duration of the study. Studying the weekly group means of P.C.V. values it is, however, surprising that mean P.C.V. values for Group 4 showed higher values than the controls for the third week to the fifth week when it dropped to a lower level on the sixth week and gradually recovered by the seventh week.

This behaviour is attributed to the occurrence of

FIG 3. EXPT. 1

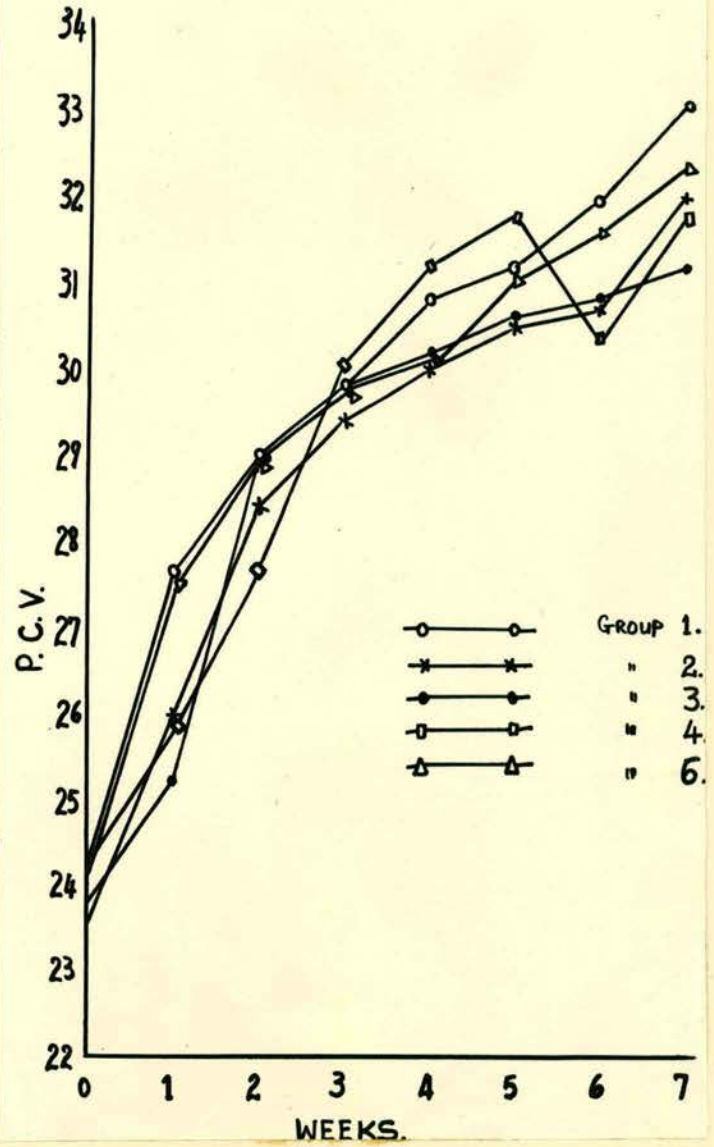


FIG 4. EXPT. 1.

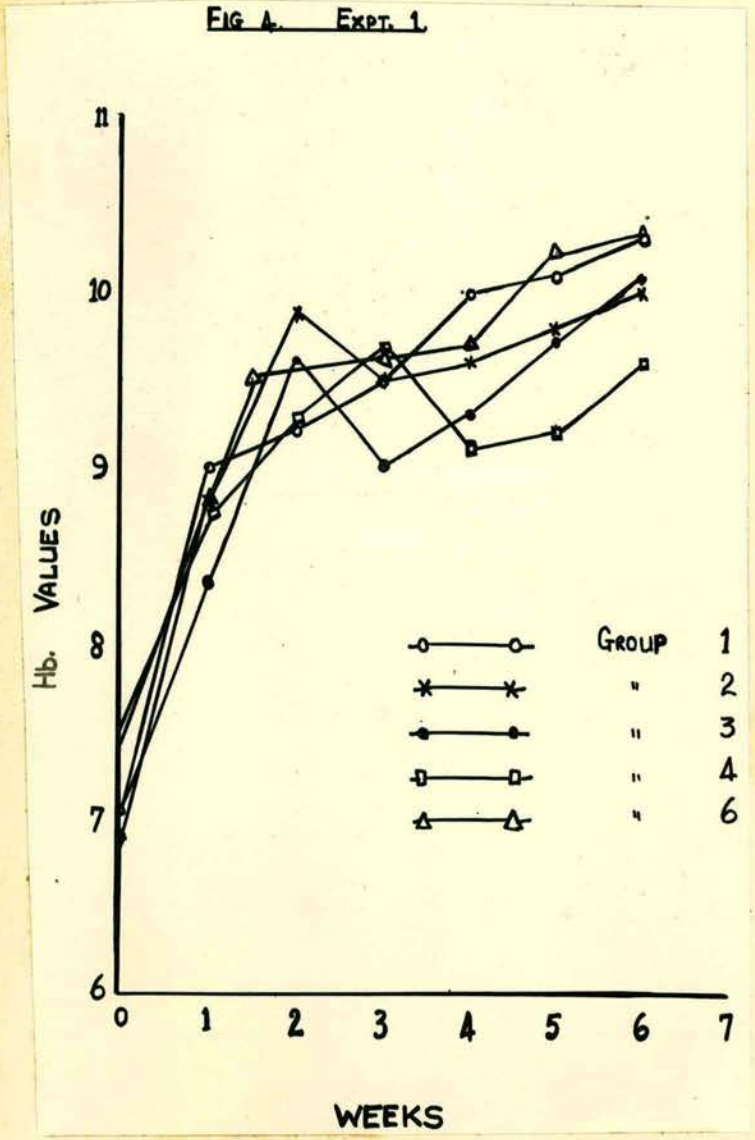


Figure 3: Packed cell volumes; weekly group means.

Figure 4: Haemoglobin values; weekly group means.

diarrhoea in chickens in this group and a continuous water loss caused the haemo-concentration seen here. With the abatement of the diarrhoea by the fifth week, recovery of normal blood volume took place, and the slight dip in the P.C.V. values could be attributed to over-compensation.

In spite of the fluctuation of the P.C.V. values occurring in this group, the analysis of variance showed no significant differences between the groups, and as such one must conclude that the infections of the order used here have no measurable effect on P.C.V. and haemoglobin values of infected chickens.

Ackert (1946) found the average haemoglobin of chickens given a moderate dosage of 200 embryonated eggs higher than that of control chickens after a period of two weeks although the figures were not significant.

Sadun (1950), working on haematocrit values, found that the average P.C.V. for a heavy single dose of 14,000 infective eggs, after ten days was less than the controls and was still so after twenty days. The average figures anyway were not significant. In the present experiment, however, high P.C.V. values due to haemoconcentration were seen. Under repeated infection of 10 eggs a day and single infection of 1000 eggs, the effect was negligible. The haemoglobin values of infected chickens showed more variation in their weekly values and seem not to bear much relation to the

diarrhoea period. The lowest values occurred on the fourth week and were seen in the group under repeated infection with 1000 eggs per day. The differences in concentration between various groups never approached significance on any of the weeks during the experiment.

Although Sadun (1950) had haematocrit average readings (individual readings were not given) which were non-significant, he nevertheless concluded that during the period of larval penetration of the intestinal mucosa there was a slight anaemia in chickens with heavy infection. In this experiment it was difficult to assess the results because of haemoconcentration. The presence of mild anaemia might be inferred by reason of the sudden drop in the mean P.C.V. values as soon as the diarrhoea stopped, or, as mentioned before, this drop could be due to over-compensation of blood volume with recovery from the diarrhoea.

Pre-patent period

Salt flotation was carried out at two-day intervals from the twenty-eighth day of infection to study the onset of egg production. In Groups 2, 5, 6 and 7 the pre-patent period was approximately thirty-six days. Eggs were not found in Groups 3 or 4 up to the eighth week when examination was stopped.

TABLE 2. EXPERIMENT 1.

Adult Worm Burdens

Group	Birds	Worm Burden	Average length
2	413	14	10.2 cm
	414	2	10.1 "
	415	3	10.3 "
	416	3	10.2 "
	417	4	9.0 "
5	424	-	-
	425	13	10.3 cm
	426	3	8.6* "
	427	-	-
6	419	2	10.5 cm
	420	7	10.7 "
	421	1	9.5* "
	422	9	11.5 "
	423	-	-
7	428	12	10.5 cm
	429	10	9.4 "
	430	14	10.1 "
	431	4	10.2 "

* Only male worms seen.

Post-mortem results

On the termination of the P.C.V. and haemoglobin measurements, the chickens were continued on their 15 per cent protein ration till 18 weeks of age and then slaughtered. The idea was to establish preliminary information about the populations of adult worms from repeated infection in comparison with single infections.

Groups 3 and 4: No birds in these groups were seen to harbour adult worms at the date of autopsy.

Groups 2, 5, 6 and 7: Birds in these groups harboured fully developed adult worms, the range per bird lying between 1 and 14 worms. These chickens, however, looked much healthier than those of Groups 3 and 4 which harboured no adult worms. The summary of the worm counts is shown in Table 2.

There were no significant differences in worm numbers or size respective to the experimental groups from which they were recovered. From this it is evident that in single dosage infections the percentage of the worm population reaching maturity is inversely related to the dosage rate.

Experiment 2

This was a preliminary experiment to study the effects of varying protein concentration of feed on the dynamics of populations of A. galli at repeated infection of 10, 100 and 1000 eggs per day and to study the effects of this infection on the chickens. The points studied were:

1. Effects of the infection and diet on weights of infected chickens.
2. Pre-patent period of the infection.
3. Counts of worm-eggs.

Experimental diets containing different protein levels - 15 per cent and 12.5 per cent and 10 per cent plus minerals - were prepared as described in Materials and Methods. The 10 per cent protein diet had no vitamin supplements. Four birds were used in each group and the experimental design was as shown below.

Group	Egg Dose	15% Protein (A)	12.5% Protein (B)	10% Protein (C)
1	Controls	4	4	4
2	R.I. 10	4	4	4
3	R.I. 100	4	4	4
4	R.I. 1000	4	4	4

Feeding of infective eggs was continued daily for six weeks. After 28 days of infection, flotation examination of faeces was done every other day to

determine the onset of egg-production in various groups.

Unfortunately the course of development of chickens fed on a low protein diet of 10 per cent, consisting of pure maize mash supplemented with phosphates and calcium, was very poor. Many deaths occurred and the experiment had to be abandoned. A high mortality also occurred in chickens fed on 12.5 per cent protein in which pure maize was used in mixing with 15 per cent protein diet to adjust the protein content to the desired level. The cause of the deaths could be the low vitamin content of maize mash coupled with the tendency of chickens housed together under such conditions of nutrition to develop severe cannibalistic tendencies. It was noticed that chickens showing signs of weakness were promptly pecked to death even in the control groups. In the next experiment, planned solely on 10 per cent protein, two things were done to offset the causes of these deaths without loss of information.

Experiment 3

To prevent the mortality just described above, the cages used for housing the chickens were partitioned as described in Material and Methods. This controlled loss of chickens due to pecking. Secondly, two types of dietary materials were prepared for the experiment, one (A) containing a vitamin supplement (Eves 9TE) as well as mineral salts, and the other (B) containing just pure maize mash without a vitamin supplement but with salts added. The experimental plan was as shown below:

Group	EggDose/Day	(A) 10% Protein + Vitamin Suppl.	(B) 10% Protein No Vitamin Suppl.
1	Controls	6	6
2	10	6	6
3	100	6	6
4	1000	6	6

From this experimental plan the interaction between the infection and diet on the chickens as well as on the worm population could be studied.

A total number of 48 birds were used, divided into 8 groups of 6 birds each. The experiment was started when the chickens were 17 days old and the repeated dosage continued for a period of six weeks.

The following data were collected:

1. Weight changes in infected chickens.

TABLE 3. EXPERIMENT 3.

Individual Weekly Weights of Chickens on 10% Protein Diet plus Vitamin Supplements

Group	Chickens	Weight at Infection	Week 1	2	3	4	5	6	7	8
1	492	136	118	145	158	155	163	171	159	165
	493	130	117	135	140	125	132	145	131	132
	494	137	130	149	167	166	167	163	148	150
	495	129	110	133	139	155	150	150	150	118
	496	114	105	126	147	138	138	140	139	160
	497	125	130	129	142	162	156	153	144	154
2	474	139	131	146	126	131	110	101	91	D
	475	133	130	133	125	124	145	132	125	126
	476	126	131	138	131	128	132	146	141	140
	477	123	105	109	114	107	107	114	120	117
	478	136	132	133	122	120	122	117	98	D
	479	117	103	112	120	120	134	142	138	129
3	480	149	135	142	153	147	142	140	154	144
	481	137	119	114	115	113	120	123	124	109
	482	128	129	131	126	130	127	124	109	D
	483	111	105	121	121	126	D	-	-	-
	484	126	116	119	118	110	112	D	-	-
	485	106	104	114	113	115	120	130	132	115
4	486	113	157	109	123	117	133	136	137	146
	487	148	138	130	130	112	D	-	-	-
	488	116	110	110	115	99	114	113	109	114
	489	116	115	118	130	120	143	132	135	136
	490	118	105	104	93	93	70	D	-	-
	491	136	122	126	129	135	152	152	150	153

TABLE 4. EXPERIMENT 3.

Individual Weekly Weights of Chickens on 10% Protein Diet without Vitamin Supplements

Group	Chickens	Weight at Infection	Week 1	2	3	4	5	6	7	8
1	328	144	144	154	151	151	154	147	139	122
	329	149	151	164	172	168	182	177	165	150
	330	114	110	113	128	129	125	127	114	95
	331	119	107	122	132	141	143	134	113	D
	332	111	107	125	133	137	120	109	D	-
	333	114	105	106	110	127	119	109	102	90
2	498	145	131	134	140	121	122	128	150	136
	499	145	124	137	143	132	125	137	145	139
	500	141	121	135	146	124	115	113	121	101
	864	126	120	114	113	116	101	103	94	D
	233	139	136	135	146	120	127	139	140	120
	227	118	106	102	110	101	81	91	90	177
3	316	132	116	126	138	144	149	139	134	101
	317	115	104	117	117	128	121	114	99	D
	318	131	125	114	127	129	136	148	132	122
	319	128	113	122	126	133	142	148	152	128
	320	144	135	128	142	133	143	150	153	142
	321	125	120	140	139	154	175	169	159	155
4	322	149	131	130	145	140	137	142	147	138
	323	149	135	116	111	95	D	-	-	-
	324	129	114	141	138	143	145	149	151	149
	325	149	144	151	149	151	D	-	-	-
	326	114	110	107	105	108	106	106	99	91
	327	110	100	90	75	D	-	-	-	-

2. Pre-patent period of infection.
3. Intensity of egg production by worms from the various groups of chickens.

Blood was taken from these chickens on the fourth week and at the end of the experiment and the serum was stored at -81°C for future use. Food and water were given to the chickens ad lib. Worm counts were done on the birds which died during the experiment and on the survivors which were slaughtered at the end of the experiment. Materials for histological sections were also obtained at post-mortem.

Results

A. Weights

The results here are interesting in that while significant weight differences were recorded between groups of chickens on type A (vitamin supplemented low protein) diet, no significant differences were noted between the groups on type B diet. Tables 3 and 4 show the individual weekly weights throughout the period of experiment, and Figures 6 and 7 show the group means for vitamin supplemented and non-supplemented birds respectively. Although both groups of chickens show poor growth, those on type A diet tended to grow more than those on type B diet especially the controls and hence the effect of infection on growth was more evident in them.

B. Pre-patent period

The pre-patent periods showed a close similarity to preliminary observations. Thus we find:

Group	Pre-patent period
2A	36 days
3A	42 days
4A	-
2B	36 days
3B	36 days
4B	42 days

The pre-patent period in the groups which received ten eggs daily was the same irrespective of dietary differences. The pre-patent period was around 36 days in both vitamin-supplemented and non-supplemented groups and was the same at the 10 per cent protein level here as in experiment 1 where 15 per cent protein diet was given to experimental chickens. Under low nutrition without vitamin supplements the pre-patent period found in Group 3B was also 36 days approximately whilst those in Groups 3A and 4B were around 42 days. In Group 3A, however, only one chicken was found to pass out worm eggs. Chickens in Group 4A passed no eggs throughout the duration of the experiment. The significance of varying pre-patent periods will be discussed in more detail later.

Figure 5: Egg counts: weekly group means.

Figure 6: Weekly group mean weights of chickens on diet with 10% protein plus vitamin supplements. Figures indicate numbers surviving in each group.

Figure 7: Weekly group mean weights of chickens on diet with 10% protein without vitamin supplements. Figures indicate numbers surviving in each group.

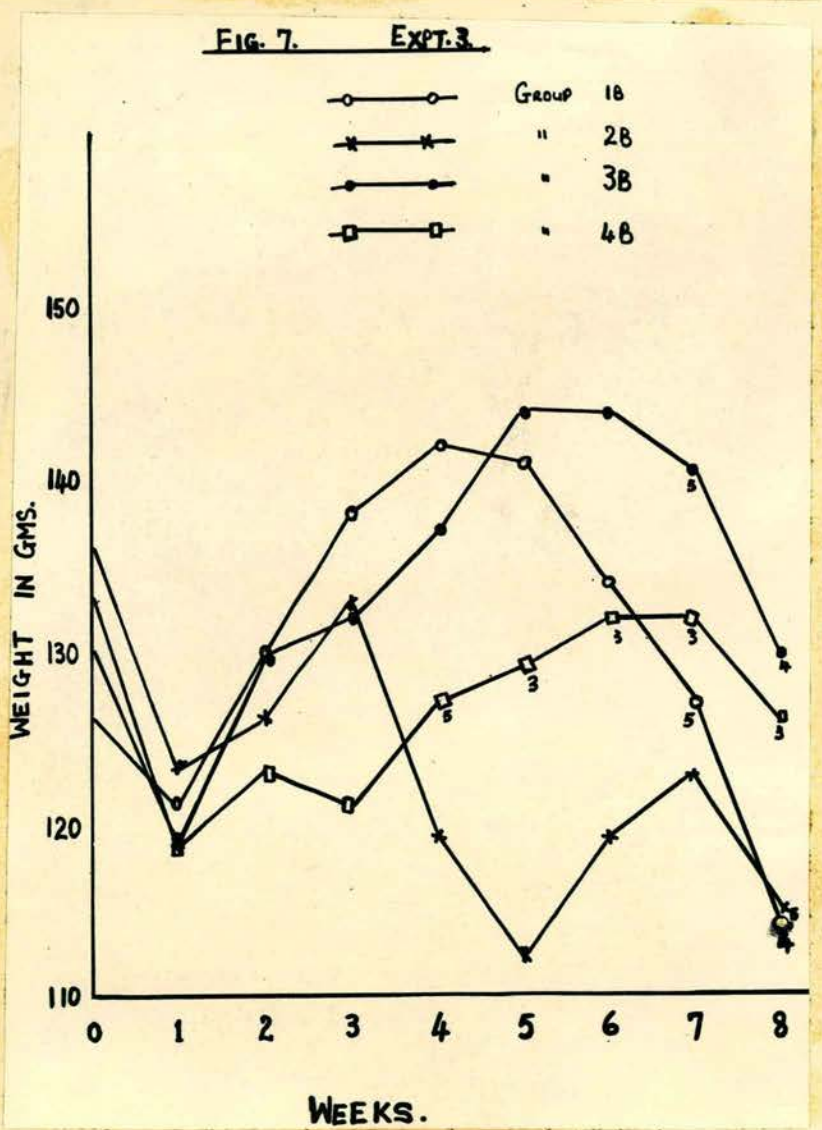


TABLE 5. EXPERIMENT 3.

Individual Egg Counts at Weekly Intervals

Grp	Chicken	(A) Low Protein plus Vitamin Supplements WEEK 1	2	3	4	Chicken	(B) Low Protein without Vitamin Supplements WEEK 1	2	3	4
2	474	4900	4000	D———		498	6700	7800	42300	11000
	75	520	1300	5200	8400	99	1800	640	700	D
	76	3350	3100	7000	6200	500	9500	7350	12000	24000
	77	4800	9850	14300	15000	864	D———			
	78	3200	6000	D———		233	600	400	1200	D
	79	650	8900	14900	20550	227	2450	4500	7400	7800
3	480	-	-	-	-	316	400	800	2000	2500
	81	-	-	-	-	17	100	2400	D	-
	82	-	1000	D———		18	1200	1050	5100	4800
	83	D———				19	1600	3000	4900	6400
	84	D———				20	1600	1400	12000	13800
	85	-	-	-	-	21	-	-	300	400
4	486	-	-	-	-	322	-	1500	7400	1700
	87	D———				23	D———			
	88	-	-	-	-	24	-	1600	4500	4000
	89	-	-	-	-	25	D———			
	90	-	-	-	-	26	-	1000	4900	D
	91	-	-	-	-	27	D———			

C. Egg counts (Table 5)

Under dietary type A only chickens which received 10 eggs per day produced high egg counts. In chickens of Group 3A only one developed a patent infection before it died; the rest in this group as well as those in Group 4A produced no worm eggs. Under the non-vitamin-supplemented diet all the experimental groups had high egg counts. The highest average counts occurred in chickens dosed with 10 eggs per day, followed by chickens receiving 100 eggs per day. The lowest counts were in chickens receiving 1000 eggs per day. The graph shown in Figure 5 summarises the group mean egg counts at weekly intervals for each group. It is probable that at least one more of the chickens in Group 3A would have passed eggs some time after the termination of the experiment for post-mortem in the eleventh week after infection eight young adults were seen, and in other chickens there were larvae in the fourth stage of development. On the other hand, in the B groups where all the birds were passing eggs before death, many young adults and advanced larvae were found post-mortem. Fewer third-stage larvae were found in groups B than in groups A although this was not precisely confirmed by differential larval counts.

D. Post-mortem results [TABLE 6]

All the birds which died during the course of the

TABLE 6. EXPERIMENT 3.

Worm Counts post-mortem

(A) Low Protein plus Vitamin Supplements						(B) Low Protein without Vitamin Supplements				
Grp	Bird	Total Adults	Young Adults	Larvae	Wk. of P.M.	Grp	Bird	Total Adults	Young Adults	Larvae	Wk. of P.M.
2	474	29	11	200	7	2	498	44	7	-	8
	75	78	41	-	9		99	15	13	100	8
	76	29	15	-	9		500	28	13	200	8
	77	49	25	-	11		864	-	-	100	6
	78	25	6	100	7		233	203	200	-	8
	79	95	10	-	11		227	100	40	-	8
3	480	-	-	400	9	3	316	7	-	1000	8
	81	-	-	300	9		17	51	31	800	7
	82	18	16	300	7		18	105	69	300	9
	83	-	-	400	4		19	170	97	400	9
	84	-	-	800	5		20	112	80	400	8
	85	8	8	500	8		21	40	28	400	8
4	486	-	-	3100	11	4	322	16	4	5000	8
	87	-	-	1500	4		23	-	-	1700	4
	88	-	-	3300	11		24	237	146	3500	9
	89	-	-	2200	9		25	-	-	1500	4
	90	-	-	1800	5		26	64	40	4100	8
	91	-	-	3600	11		27	-	-	1000	3

experiment were opened up and examined. The five birds of Groups A which survived were slaughtered at the end of the eleventh week and examined also. The adult worms were counted directly, and the larvae digested out with pepsin and their numbers estimated by dilution sampling. No adult worms were found in any of the birds until the seventh week. Of the chickens which survived longer than this all those receiving the lowest challenge (10 eggs per day) contained substantial numbers of adults ranging from 25 to 95 in the vitamin-supplemented group (2A) and from 15 to 203 in the non-supplemented group (2B). At the challenge rate of 100 eggs per day, only two out of six birds had adult worms in Group 3A and the infestations were small (8 and 18 adults), while in the vitamin-deficient group (3B) all the birds were infested with adult worms in substantial numbers varying from 7 to 170. At the high challenge rate there was a complete contrast between the two dietary groups for while the vitamin-supplemented group (4A) contained no adults in any of the six birds, the deficient group had 3 out of 6 infested birds, namely, those that survived beyond four weeks. The relations between the six groups are thus systematic. Vitamin-deficient groups all develop adult worms irrespective of the challenge rate, while vitamin-sufficient birds even at this low protein level show an increasing ability to inhibit adult development as the challenge rate increases.

Larvae were not identified in the two birds of Group 2A which survived up to 11 weeks nor were they seen in the two birds of this group which died at 9 weeks. They were observed in three out of the six birds from Group 2B which died at 8 weeks. Larvae were observed in all birds receiving a higher challenge than 10 eggs per day and were still present as late as 11 weeks in the three survivors of Group 4A.

In this experiment all the groups that showed adult worms had both young and adult worms present which showed that continued development was going on.

Experiment 4

This experiment is a repeat of experiment 2 modified in the light of the results obtained from the last experiment. Here under two dietary protein levels of 15 per cent and 12.5 per cent, the birds were infected with doses of 10, 100 and 1000 infective eggs. Data were collected on:

1. Weights of infected chickens.
2. Pre-patent period of infection.
3. Egg counts when possible.

The experimental plan is similar to that used in the last experiment. The infection was continued daily for six weeks. At the end of 30 days examination of faeces for eggs was carried out every third day. At the end of the experiment blood was taken from the chickens and the sera stored at -81°C for later use. Weekly weight measurements were continued until the eighth week, and egg counts were continued for only four weeks. Forty-eight chickens were arranged in eight groups of six and the groups numbered and treated as in the following table:

Eggs/Day	Group	(A) 15% Protein	(B) 12.5% Protein
Control	1	6	6
10	2	6	6
100	3	6	6
1000	4	6	6

TABLE 7. EXPERIMENT 4.

Individual Weights at Weekly Intervals of Chickens on 15% Protein Diet

Group	Chickens	Weight at Infection	Week 1	2	3	4	5	6	7	8
1	334	76	119	159	238	280	372	514	574	666
	335	99	140	205	285	356	495	654	727	793
	336	102	138	175	279	343	410	519	582	657
	337	100	119	169	248	283	336	470	524	616
	338	98	121	180	268	330	378	545	675	794
	339	80	120	165	239	300	360	464	507	601
2	340	87	125	144	214	280	361	451	522	584
	341	104	148	152	242	356	449	546	585	646
	342	84	128	149	220	275	335	425	445	513
	343	84	128	152	213	281	343	479	554	670
	344	83	125	153	214	286	364	449	562	668
	345	78	121	144	221	300	389	506	587	683
3	346	101	147	169	242	260	326	492	570	681
	347	78	112	129	205	253	334	540	532	595
	348	109	139	136	217	241	372	513	546	696
	349	82	93	126	166	194	282	367	452	491
	350	85	122	161	163	249	297	421	419	480
	351	89	127	141	179	214	293	496	500	508
4	352	76	95	123	143	156	206	260	288	309
	353	104	127	139	189	207	293	382	455	613
	354	100	127	139	198	250	324	438	455	505
	355	89	119	152	184	203	279	390	477	583
	356	80	107	130	180	276	346	480	508	578
	357	82	105	122	156	179	253	344	370	373

TABLE 8. EXPERIMENT 4.

Individual Weights at Weekly Intervals of Chickens on 12.5% Protein Diet

Group	Chickens	Weight at Infection	Week 1	2	3	4	5	6	7	8
1	358	96	100	172	215	248	316	379	469	338
	359	82	113	143	177	217	272	318	393	459
	360	103	115	141	182	214	270	338	391	445
	361	82	106	141	172	190	257	314	384	448
	362	95	122	153	198	225	315	351	400	480
	363	79	100	142	188	217	312	382	433	530
2	364	89	114	151	180	226	304	370	430	459
	365	94	119	130	200	223	300	364	407	519
	366	103	133	154	220	239	280	373	433	524
	367	91	117	143	220	256	320	313	457	530
	368	86	122	127	203	238	294	321	372	509
	369	86	109	143	194	206	262	333	378	425
3	370	108	140	164	194	197	282	345	412	492
	371	82	115	128	162	195	240	295	380	422
	372	92	118	124	155	173	215	252	304	347
	373	96	132	156	187	208	267	385	475	575
	374	89	119	150	194	236	292	371	446	514
	375	86	110	100	122	138	195	239	280	324
4	376	80	102	120	125	109	173	100	D	-
	377	76	105	96	183	201	217	261	301	348
	378	108	127	149	193	210	309	392	469	560
	379	101	118	140	172	200	274	212	397	484
	380	86	115	131	168	170	252	326	350	386
	381	81	103	133	141	134	224	270	300	364

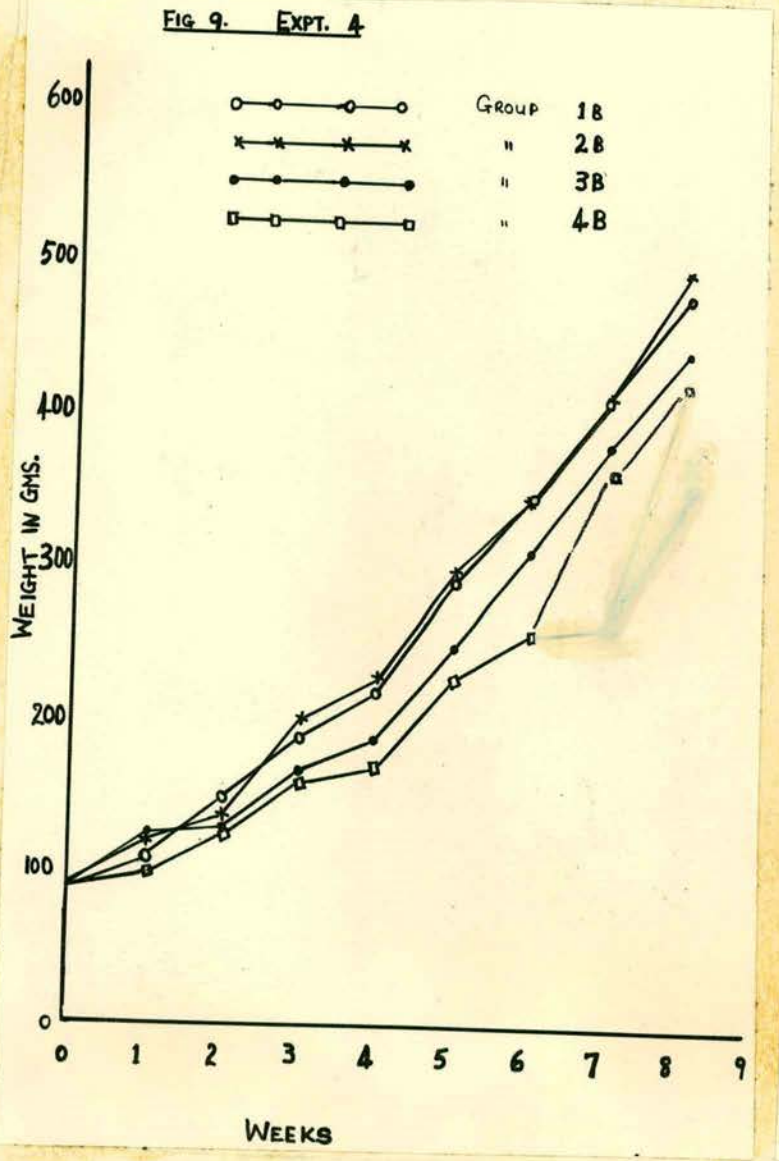
Results

Tables 7 and 8 show the actual weights of each chicken, and Figures 8 and 9 the weekly mean weights of the groups for the duration of the experiment. In this experiment the food was more nutritious and all the chickens grew. There were highly significant progressive weight differences starting from the second week of infection between the four groups on the 15 per cent protein diet.

The chickens, both controls and infected, on a 12.5 per cent protein diet did less well than chickens on 15 per cent protein. However, significant weight differences again occurred between the groups. This developed by the third week and persisted up to the fifth week.

Throughout the experiment the chickens under 15 per cent protein diet as would be expected had a growth advantage over those under a 12.5 per cent protein diet. Also in both dietary groups the growth rate was always in favour of the groups under a repeated infection of 10 eggs per day as compared with the chickens under daily dosage rates of 100 and 1000 eggs. The chickens receiving 1000 eggs per day always made the poorest weight gains.

The control chickens under a 12.5 per cent diet did less well than might be expected in comparison with chickens fed 10 eggs daily although the weight differences



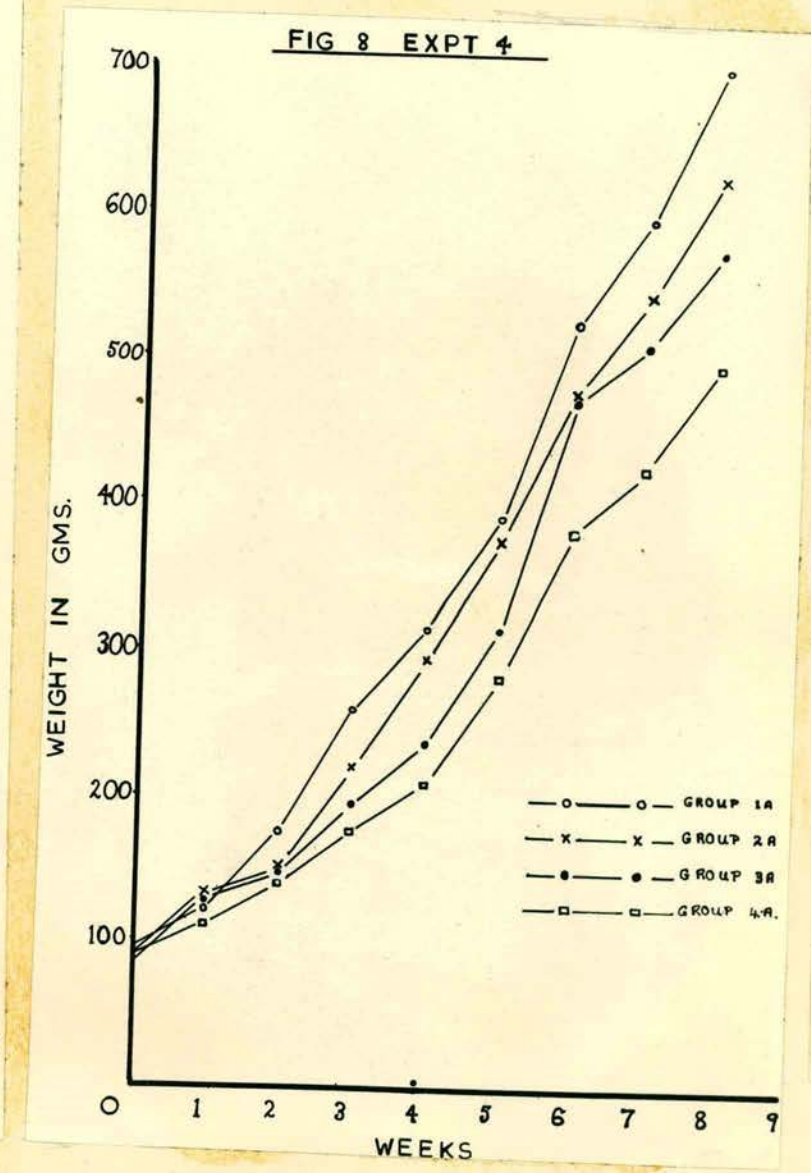


Figure 8: Weekly group mean weights of chickens on 15% protein diet.

Figure 9: Weekly group mean weights of chickens on 12.5% protein diet.

were not significant. On the other hand, control chickens on 15 per cent protein showed marked differences in weight gains from chickens infected with a daily dose of 10 eggs per day. This was also the case in the previous experiment when the birds were on a 10 per cent protein diet with supplemented vitamins and those infected with 10 eggs per day gained less weight than the controls.

During the experiment no possible explanation for the control group under 12.5 per cent protein diet doing "badly" in comparison with chickens under a repeated infection of 10 eggs a day could be found. It might be possible that the movement of the controls into batteries contributed to this because it had been noticed previously that removal of chickens often upsets their appetite for a couple of days before they become used to their new surroundings. On the other hand, birds recovering from infection and harbouring adult worms have been reported to feed voraciously for some time (Roberts, 1937) and this might serve to explain the apparent discrepancy of the growth rates seen here.

Pre-patent period of infection

In this experiment the pre-patent period again showed close similarity to those in previous experiments as far as the groups receiving repeated infections of 10 eggs per day were concerned. This was irrespective of

TABLE 9 : EXPERIMENT 4

INDIVIDUAL EGG COUNTS AT WEEKLY INTERVALS

GROUP 2									
	15% PROTEIN DIET					12.5% PROTEIN DIET			
Birds	1	2	3	4	Birds	1	2	3	4
340	-	-	-	-	364	250	400	1,300	1,600
341	200	1,300	3,800	250	365	400	7,000	1,550	950
342	550	900	2,350	2,450	366	200	550	1,600	800
343	200	600	800	1,050	367	100	800	1,950	1,700
344	100	500	550	2,500	368	-	-	-	-
345	-	-	-	-	369	1,400	900	2,950	3,850

the protein concentration of diet and was around 36 days. Other groups, however, showed no egg-counts, except for one bird in Group 3B where a pre-patent period of 61 $\frac{16}{\wedge}$ $\frac{64}{\wedge}$ days was obtained.

Egg counts

The egg counts of chickens given a repeated infection of 10 eggs a day are shown in Table 9. They all showed moderately heavy egg counts throughout the duration of the experiment irrespective of the percentage of the protein diet they were put on. The average counts from the chickens with the lower protein diet were only slightly higher than the counts from chickens on the 15 per cent protein diet, but the differences were not significant. After 64 days from the beginning of the infection one chicken in Group 3B was passing out eggs. Only one egg count was done before slaughter and this showed 850 eggs per g. of faeces.

Post-mortem results

After 10 weeks from the beginning of the experiment, three birds from each group were slaughtered and the intestines digested for worm counts. The results are shown in Table 10.

At the sixteenth week after infection, the remaining chickens on 12.5 per cent protein diet were slaughtered and subjected to the same treatment as above. The

TABLE 10 : EXPERIMENT 4

WORM COUNTS POST-MORTEM

[A] 15% PROTEIN DIET						[B] 12.5% PROTEIN DIET					
GRP	Bird	Total Adults	Young Adults	Larvae	Week of pm	GRP	Bird	Total Adults	Young Adults	Larvae	pm wk
	340	-	-	-	10		364	12	-	-	10
2	341	12	-	-	10	2	365	2	-	-	10
	345	-	-	-	10		366	8	-	-	10
	346	-	-	200	10		373	2	2	200	10
3	347	-	-	400	10	3	374	5	5	600	10
	348	-	-	100	10		375*	5	4	200	10
	352	-	-	300	10		376	-	-	1,400	6
4	353	-	-	800	10	4	377	1	1	400	10
	354	-	-	400	10		378	-	-	700	10

12.5% PROTEIN DIET					
GRP	Bird	Total Adults	Young Adults	Larvae	Week of Post.M
	367	4	-	-	16
2	368	-	-		16
	369	1	-	-	16
	370	60	40	-	16
3	371	3	-	-	16
	372	35	13	-	
	379	10	8	-	16
4	380	-	-	-	16
	381	3	2	200	16

* 375 Egg laying - 850 eggs per gram. of faeces.

remaining chickens receiving 15 per cent protein diet were retained for further observations on age and acquired resistance.

The post-mortem results showed no larval population after 10 weeks in the groups fed 10 eggs per day (2A and 2B). All worms recovered from them were adults. Chickens from Groups 3A, 4A, 3B and 4B contained larvae exclusively in the A groups, and numerous larvae together with a few adults of which one only was mature in the B groups.

The post-mortem examination carried out after 16 weeks of infection was on experimental groups 2B, 3B and 4B. Chickens of Group 2B showed no significant change from the findings in birds of the same group to those examined post-mortem after 10 weeks. Again only adult worms were recovered. Similarly although several adults were recovered from chickens in Group 3B no larvae were now seen; while a fair proportion of these adults was still immature, mature adults were also present in all the chickens. In chickens in Group 4B the results were similar to Group 3B, but one still harboured larvae, whilst others had both egg-laying and young adults.

These results show that although adults occur in all groups, their development is much delayed. Although flotation examination of faeces showed quite a heavy egg-output it was not practicable to carry out detailed

counts. The resultant effect of the slow development is the occurrence of a long pre-patent period, varying from chicken to chicken. The significance of these findings in relation to resistance and epidemiology will be discussed later.

Experiment 5

This experiment was devoted mainly to the study of the effect of age and prior experience of infection on the development of parasite populations. Previous studies summarised in the Introduction have based their assessment of the presence or absence of age- and acquired-resistance on larval counts in infections of only three weeks duration. Results as obtained from such experiments, whilst perhaps being satisfactory from the point of view of larval populations, become entirely inadequate when one wishes to relate the phenomena of immunity and acquired resistance to adult egg-laying worm populations. Moreover, they are meaningless in the relation to the use of egg-output as a guide for predicting the margin of safety which susceptible chickens can be accorded when in contact with those having a prior experience of infection or displaying age resistance.

The planning of the experiment took advantage of the birds remaining from experiment 4. Seven groups were used, and included three chickens from each of Groups 1A to 4A (15 per cent protein diet) of experiment 4, three two-week old chickens, three fourteen-week old chickens and three twenty-two-week old chickens.

The chickens from the previous experiment were dosed with Safersan to remove any worms present. One week after the dose all the chickens were given a single

infection of 1000 embryonated eggs and fed on a diet of 15 per cent protein throughout the duration of the experiment.

To study the onset of egg production faecal examinations were carried out every third day after 30 days.

Results

Pre-patent period

Group 1 (1A of experiment 4)	36 days
Group 2 (2A of experiment 4)	36 days
Group 3 (3A of experiment 4)	36 days
Group 4 (4A of experiment 4)	36 days
Group 5 (2 week old)	36 days
Group 6 (14 week old)	51 days
Group 7 (22 week old)	No eggs passed

If we compare the last three groups (viz. 5, 6 and 7) which include the newcomers aged two weeks, 14 weeks and 22 weeks, there is a clear direct relationship between the length of the pre-patent period and age. It is shortest in the youngest birds (36 days), longer in the older birds of 14 weeks of age (51 days) and infinite in the oldest birds (i.e. the infection never became patent). There is thus clear evidence of an inhibition of development of worms to patency increasing with the age of the host where a first experience of infection is concerned. On the other hand, these

TABLE 11 : EXPERIMENT 5

AGE AND ACQUIRED RESISTANCE: EGG COUNTS
AT WEEKLY INTERVALS AND WORM COUNTS POST-MORTEM

GRP	Chicken	Wk. 1	2	3	4	5	No. of worms post-mortem	pm wk
	336	-	-	-	-	-	-	11
1	337	100	750	6600	900	750	12	11
	339	100	100	500	350	150	4	11
	342	100	300	300	400	200	4	11
2	343	550	200	150	150	100	9	11
	344	-	-	-	-	-	-	11
	349	400	500	200	50	350	3	11
3	350	50	200	100	150	100	2	11
	351	800	150	250	150	450	4	11
	355	-	50	50	150	250	1	11
4	356*	-	-	D	-	-	[300 larvae]	7
	357	-	200	50	100	350	2	11
	1	400	100	550	1,000	1,200	7	11
5	2	800	200	700	960	840	4	11
	3	500	500	1,200	1,400	960	10	11
	45	-	-	200	50	50	2	11
6	46	-	-	-	100	100	2	11
	47	-	-	200	150	150	2	11
	120**	-	-	-	D	-	[167 larvae]	9
7	121	-	-	-	-	-	-	11
	122	-	-	-	-	-	-	11

* DIED 7th WEEK

** DIED 9th WEEK.

results contain no evidence that previous repeated challenge enhances resistance to subsequent challenge infection as measured by the pre-patent period.

Only one out of the nine birds with previous experience proved refractory to infection. The other eight became infected and the pre-patent period was no longer than the pre-patent period of Group 1 chickens (non-infected controls of previous experiment) or of the much younger birds of Group 5 (2 weeks old). Nevertheless, these birds were 12 weeks old and all of them, whether previously infected or uninfected, had much shorter pre-patent periods than the birds in Group 6 which were only 2 weeks older.

This difference might be attributed to the effect of anthelminthic treatment prior to the infection. This might have had the effect of reducing any resistance they might have developed due to a previous infection as well as removing the worm burden. On the other hand, it might be possible that a previous repeated infection makes no difference to the development of resistance to a challenge infection as far as the length of the pre-patent period is concerned.

The egg-output (Table 11) in this experiment does not really show much from which one could argue for or against the presence of resistance due to previous infection or age. It is, however, evident from the results that egg output in the two-week old chickens is slightly higher

than in the other groups. Also there was no egg output from the chickens aged 22 weeks throughout the duration of the experiment.

Chickens from experiment 4 which had received prior infections of varying repeated dosages do not seem to show differences in their egg output which can be attributed to this. It is quite possible that significant results might have been obtained had more chickens been used. One chicken from each of the Groups 1 and 2 passed out no eggs and had no worms or larvae.

Evidence from the numbers of worms recovered post-mortem tend to indicate a slight resistance due to previous infection and age. Whilst chickens infected at the age of 2 weeks harboured an average of 10.5 worms per chick, chickens given a prior experience of repeated infection of 10, 100 or 1000 eggs daily for 6 weeks (i.e. Groups 2 to 4 inclusive) harboured an average of 7.5, 3 and 1.5 worms respectively. The previously non-infected birds (i.e. Group 1 or controls from experiment 4) contained an average of 8 worms.

These results tend on the whole to show that age resistance operates in chickens against A. galli infections but only a very slight resistance results from a previous repeated infection.

From the epidemological point of view these results contain less comfort for the poultry farmer than appears to be implicit in the results of workers who have

studied resistance by larval or young adult counts developing from single doses of infection after a period of about 3 weeks.

It is not possible to relate these findings to those of other workers since this appears to be the only case where an attempt is made to identify by a subsequent single challenge the possible acquisition of resistance due to previous repeated challenge at low or high levels.

SECTION 2

Experiment 6

This section deals with the results of one single experiment. Experiments 1 to 4 provided only a limited opportunity for studying the metamorphosis of worm populations from egg to adult in the chicken gut since comparative population counts were only possible at the conclusion of each experiment. These, however, supplemented by the observations on birds which died prematurely, contain some consistent indications. At a low challenge level, a substantial proportion of the population reaches the adult stage. Larvae are rarely encountered after the establishment of adult populations. At high challenge levels metamorphosis to the adult is significantly delayed and larval populations persist in readily recognisable proportions. Inhibition of larval development is most pronounced in birds with fully satisfied nutritional needs (i.e. 15 per cent protein-level diets). Reduced nutritive levels without vitamin supplement reduce the tendency but slight inhibition still occurs even at this extreme level of malnutrition. The failure to identify persistent larvae in birds which have sustained a low infective intake is not simply a result of technical incompetence, since inhibition of development at a rate comparable to those observed in the heavy challenge cases would certainly result in

TABLE 12

EXPERIMENT 6

DIFFERENTIAL WORM COUNTS OF BIRDS SLAUGHTERED AT WEEKLY INTERVALS AFTER CHALLENGE WITH 10 and 1000 EGGS PER DAY

WK	R. 10					R.1 1000				
	2nd Stage Larvae	3rd Stage Larvae	4th Stage Larvae	Total Larval Pop.	Total Adult Pop.	2nd Stage Larvae	3rd Stage Larvae	4th Stage Larvae	Total Larval Pop.	Total Adult Pop.
1	33	33	-	66	-	167	-	-	167	-
	33	-	-	33	-	133	-	-	133	-
	67	-	-	67	-	267	-	-	267	-
	33	-	-	33	-	200	-	-	200	-
	67	-	-	67	-	133	-	-	133	-
	233	33	-	267	-	900	-	-	900	-
2	-	33	-	33	-	1500	667	-	2167	-
	33	33	-	66	-	1900	200	-	2100	-
	33	-	-	33	-	667	667	-	1333	-
	100	33	-	133	-	1000	600	-	1600	-
	33	67	-	100	-	600	400	-	1000	-
	199	166	-	366	-	5667	2534	-	8200	-
3	-	33	-	33	3	1000	800	-	1800	-
	33	67	-	100	-	33	167	-	200	-
	-	33	33	66	-	633	300	-	933	-
	33	67	-	100	5	1000	500	-	1500	-
	33	-	33	66	2	1000	667	-	1667	-
	99	200	66	365	10	3666	2434	-	6100	-
4	-	100	-	100	3	167	500	-	667	-
	33	-	33	66	1	33	67	-	100	-
	-	67	-	67	-	200	300	-	500	-
	33	33	33	99	3	800	1000	-	1800	-
	-	-	-	-	3	600	333	-	933	-
	66	200	66	332	10	1800	2193	-	4000	-

	-	33	33	66	8	-	33	-	33	-
	-	67	-	67	6	33	300	-	333	-
5	-	100	33	133	3	-	933	-	933	-
	-	33	-	33	1	-	333	-	333	-
	66	33	33	132	10	-	667	-	667	-
	66	266	99	431	28	33	2266	-	2299	-
	33	-	-	33	8	-	500	-	500	-
	-	33	33	66	14	-	333	-	333	-
6	33	-	66	99	13	-	100	-	100	-
	-	33	-	33	6	-	400	-	400	-
	-	67	-	67	1	-	800	-	800	-
	66	133	99	298	42	-	2133	-	2133	-
	33	33	33	99	11	-	400	-	400	-
	-	67	-	67	22	-	500	-	500	-
7	33	-	-	33	11	-	200	-	200	-
	-	-	33	33	10	-	633	33	666	-
	-	-	-	-	8	-	400	-	400	-
	66	100	66	232	62	-	2133	33	2166	-
	-	-	-	-	0	-	500	-	500	-
	-	-	-	-	22	-	167	-	167	-
8	-	-	-	-	14	-	500	-	500	-
	-	-	-	-	10	-	833	-	833	-
	-	-	-	-	16	-	533	-	533	-
	-	-	-	-	62	-	2533	-	2533	-
	-	-	-	-	10	-	367	-	367	-
	-	-	-	-	12	-	467	33	500	-
10	-	-	-	-	6	-	233	-	233	1
	-	-	-	-	10	-	500	33	533	-
	-	-	-	-	4	-	800	-	800	3
	-	-	-	-	42	-	2367	66	2433	4
	-	-	-	-	10	-	600	-	600	-
	-	-	-	-	6	-	-	-	-	-
12	-	-	-	-	19	-	400	-	400	2
	-	-	-	-	7	-	167	-	167	-
	-	-	-	-	7	-	200	33	233	-
	-	-	-	-	49	-	1367	33	1400	2

	-	-	-	-	1	-	33	-	33	2
	-	-	-	-	8	-	200	-	200	1
16	-	-	-	-	15	-	33	33	66	-
	-	-	-	-	7	-	167	-	167	-
	-	-	-	-	10	-	67	-	67	3
	-	-	-	-	41	-	500	33	533	6
	-	-	-	-	6	-	300	-	300	3
	-	-	-	-	2	-	167	-	167	-
19	-	-	-	-	2	-	33	33	66	4
	-	-	-	-	9	-	-	-	-	1
	-	-	-	-	3	-	266	-	266	-
	-	-	-	-	22	-	766	33	799	8

R.1 1000 - Repeated Infection 1000 eggs.

R.1 10 - Repeated Infection 10 eggs.

retention of identifiable proportions of even the smallest larval populations employed in these experiments.

The present experiment was designed, therefore, to explore more closely the differences in metamorphosis rates of larvae acquired at the rates of 10 per day and 1000 per day. It was necessary to confine the observations to these levels of larval uptake (the extremes employed in the present work) to keep the experiment of manageable proportions. A total of 120 birds was used, 60 in each group. All the birds were fed on a diet containing 15 per cent protein. Five birds from each group, representing high and low egg-challenge, were killed at weekly intervals from the first to the eighth week of the experiment inclusive and thereafter on the tenth, twelfth, sixteenth and nineteenth weeks. Embryonated eggs were administered daily for the first six weeks of the experiment. Differential worm counts were performed on the slaughtered birds. Portions of the posterior region of the duodenum were removed and fixed for subsequent pathological examination. All the chickens were bled prior to slaughter and the individual sera stored at -81^{o}C for later examination.

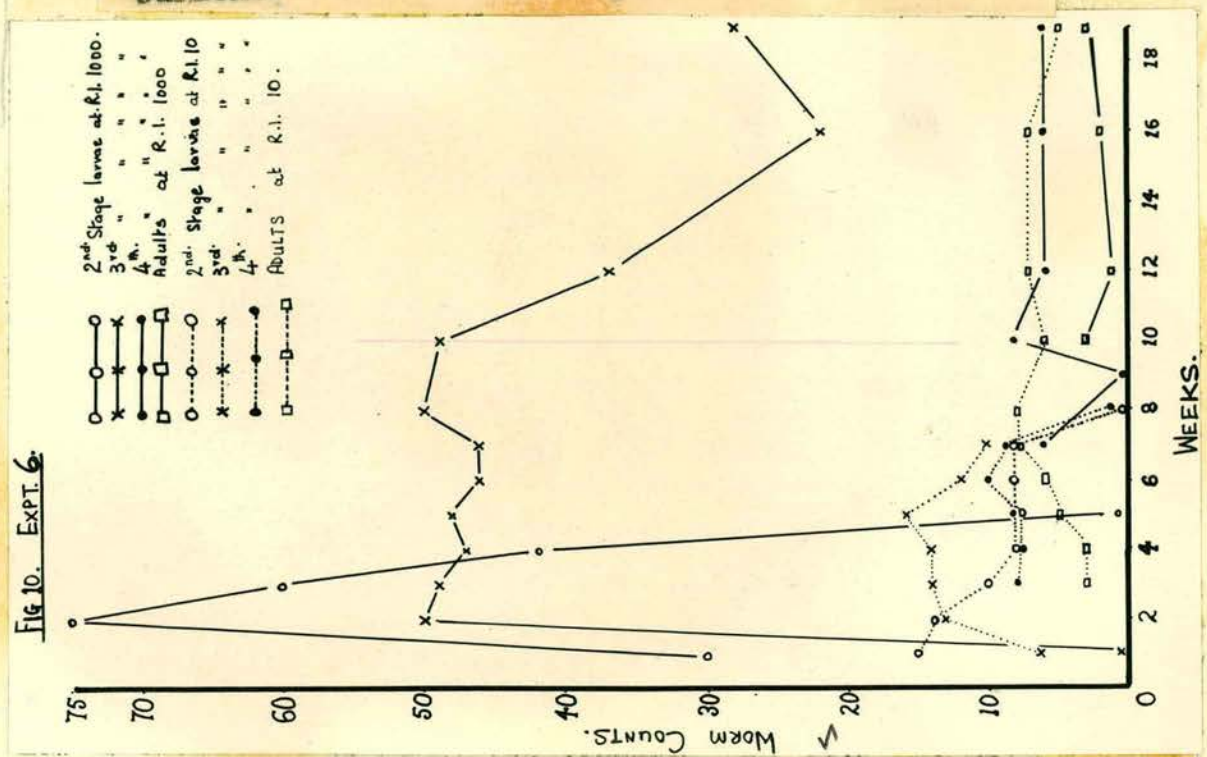
Results

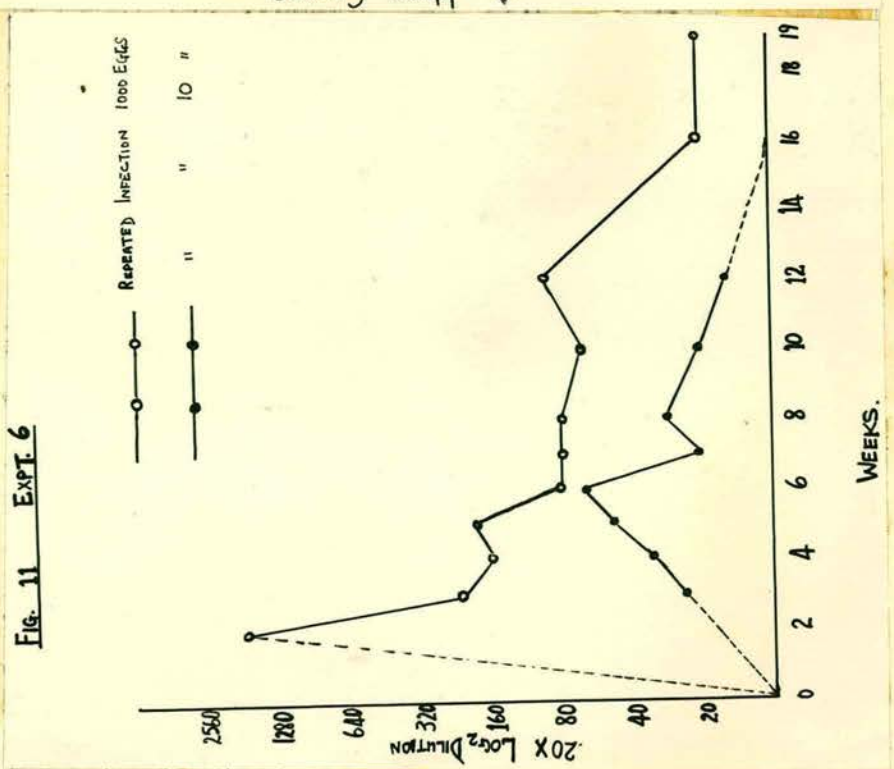
A. Differential worm counts

The findings are summarised in Table 12 and are

Figure 10: Worm counts of birds slaughtered at weekly intervals following challenge of 10 and 1000 eggs per day. The points represent the sums of each helminth stage from the 5 birds slaughtered on each occasion from each of the challenge groups.

Figure 11: Antibody titres from birds slaughtered at weekly intervals following challenge of 10 and 1000 eggs per day. The points represent the mean titre of 3 birds of the 5 slaughtered for weekly counts on worm burdens.





represented in Figure 10. The findings confirm the broad indications of earlier experiments that substantial numbers of worms reach maturity in birds receiving small infections, whereas only a very small minority of the heavy infections reach the adult stage. The difference in adult numbers is not merely relative but is absolute. Secondly the earlier findings are confirmed in that larval stages disappear soon after the cessation of low-rate challenge whereas larvae persist in substantial numbers at least up to 13 weeks after the termination of high-rate challenge.

With respect to adult worms the marked differences observed between the two groups can be accepted with full confidence since the worms are individually recognisable. In the low-challenge group adult worms were already present in three out of five birds in the third week. Thereafter, they were continuously present in all birds of this group with two exceptions only out of 45 until the experiment was terminated in the nineteenth week. Out of the 60 birds in the group 46 were infected and contained a total of 370 adult worms. As against these results the high-challenge group did not produce adult worms until as late as the tenth week and then there were only 9 birds out of the whole group, containing between them only 20 adult worms.

Fourth-stage larvae were only occasionally recovered and this fact together with the fact that enumeration is

based upon a dilution sampling technique allows only tentative conclusions in very general terms. The two groups differed in the time of appearance of larvae of this stage. In the high-challenge group they were not recognised until the tenth week, the same week in which adults were first observed in this group. In the low-challenge group, on the other hand, they had already been observed in the third week, again the same week when adults appeared in the group. In the low-challenge group, fourth-stage larvae appeared sporadically from the third until the seventh week and never thereafter. In contrast they appeared sporadically after the tenth week right up to the nineteenth week in the high-challenge group. There is good reason to accept these figures as representing the true situation since even though larvae appear but intermittently in the 1 ml aliquots on which counts were made, they occur within the expected frequency limits. Consider, for example, the low-challenge group from weeks 3-7 when fourth-stage larvae were seen. During this period 152 adults were recovered from 25 birds. Thus, over this period at least the same number of fourth-stage larvae must have occurred. Such a number would occur in the 1 ml samples at the rate of one larva per 17 samples. In fact, 12 larvae were observed in 75 samples which is approximately three times more frequently than the required minimal rate. Similarly from weeks 10-19 where the minimum expected

frequency was 1:100 in the high-challenge group, fourth-stage larvae were found in 5 out of 60 samples which is eight times the expected frequency. These figures strongly imply a mortality among fourth-stage larvae and suggest that it is greater in the high- than in the low-challenge group. We can also accept that metamorphosis from third to fourth instar is significantly delayed in the high-challenge group.

Second and third stage larvae were recovered in considerably larger numbers and the findings permit of rather more precise conclusions. In the low-challenge group these stages were found only during the challenge period and in the subsequent week. This can only mean that larvae which fail to complete metamorphosis through to the fourth and adult stages are simply lost. In the other group, where delay in the appearance of fourth-stage larvae and adults implies a retarded metamorphosis we find the expected corollary of prolonged persistence of third-stage larvae. Third-stage larvae continue to be found in substantial numbers right up to the nineteenth week. Only one conclusion is possible from this, namely, that the retarded metamorphosis which occurs in high-level infestations operates through a delay in the third to fourth moult. There is, however, a further striking feature in the third larval counts particularly evident in the high-challenge group. From the second to tenth weeks inclusive the total numbers of larvae-3 recovered

remain remarkably constant, ranging from the lowest sum of 2,133 in weeks 6 and 7 to the highest sum of 2,533 in weeks 2 and 8. The rises and falls are randomly distributed between the weeks. The distribution of counts between the individual birds reveals a variation which exceeds greatly what would be expected from random sampling of a homogeneous population ($X^2_{39} = 140.5$). Excluding the extreme counts for each week the variation between the remaining individuals becomes such that they could have been drawn from a homogeneous population ($X^2_{23} = 12.5$) in spite of the fact that they include a succession from 2 weeks up to 10 weeks after the initiation of infection. In this group second-stage larvae appeared in large numbers from the first to the fourth weeks although there is a pronounced declining trend from weeks 2 to 4. After week 4 larvae of this stage are no longer recognised in spite of the introduction of 1000 embryonated eggs within 24 hours before the post-mortem examinations of the fifth and sixth weeks. It is concluded that by the fourth to the sixth weeks even a temporary establishment of the incoming worms has been prevented and hence the whole population record from the fourth week onwards must have derived from the infection of the first three weeks. Thus the third-stage population seen from weeks 4 to 10 must be the same one since reinforcement is impossible. Similarly in the period before the fourth week there is no loss of third-stage

population through metamorphosis to stages four and beyond. If there is a population flow through the third instar earlier than week 4 it can only take place through a mortality and loss of third-stage individuals. There is no evidence for this and the conclusion follows that the whole future pattern is already determined in the first and second weeks of infection. Once established by the second week the third-stage population apparently remains static until about the tenth week when a few individuals resume development to fourth stage and beyond and the rest are slowly lost. A brief delay of metamorphosis of third-stage larvae is suggested by the figures of the low-challenge group but these figures are so small that the point cannot be emphasised with any confidence.

B. Goblet cell counts [TABLE 13]

Goblet cell counts were done on sections from three chickens per group, except in the first week when only one section from the groups under repeated infection of 1000 and 10 respectively could be used. Total counts were done under a one-sixth objective and the averages of five counts were taken. The results show an increase in the number of goblet cells with age of the chicken. This supports the finding of Ackert et al. (1939), but whether this has any function in resistance of the chickens to infection will be dealt with in another section. Increase in goblet cell counts

TABLE 13 : EXPERIMENT 6

GOBLET CELL COUNTS

Groups	At Infection	Week 1	2	4	8
Controls	88	127	124	180	261
	66	117	115	201	183
	81	132	129	132	200
R.1.1000	-	137	180	188	209
	-	-	165	185	240
	-	-	173	153	264
R.1.10	-	123	160	148	208
	-	-	125	184	198
	-	-	140	168	258
Low protein	-	-	198	204	289
Plus vit.	-	-	227	230	263
R.1.1000	-	-	188	241	258

R. I. (repeated infection)

occurred also with infection. Chickens receiving 1000 eggs daily showed more mucus cell counts, especially in the second week of infection, than those under a repeated infection of 10 or the control chickens. On comparing these counts with those from chickens on a low protein diet with vitamin supplements and receiving a daily infection of 1000 eggs, it is noticed that, throughout the period of the study, the average weekly counts were consistently much higher. These chickens harboured more larvae than chickens on 15 per cent protein diet and the presence of higher goblet cell counts might be attributed to greater irritation due to a larger larval population. Although the differences in all the counts were significant only in the second week of infection, significant differences could probably have been shown if greater numbers of chickens had been used. All the same there was no period during the duration of the count when goblet cell numbers in chickens under low protein were less than counts obtained from control chickens or infected chickens on a high protein diet. These results differ from those obtained by Wells (1963) working on Nippostrongylus brasiliensis infection of rats under single and multiple infections. She found that rats on a high protein diet had an increase in goblet cell counts in response to single infection and following multiple infections the number of mucin cells remained normal. In contrast to this, under a low protein diet she found

that a fall in the number of goblet cells occurred following both single and multiple infections. The differences obtained here will be discussed later. From this and the earlier experiments, it was noticed, however, that repeated infections especially of the order of 1000 eggs per day stimulated a rapid production of mucus-secreting cells which tended to be related to the number of worms present at the infection as shown by chickens under a low protein diet. The possibility of there being an immune response tied up to mucus secretion cannot be discounted and this will be examined in future experiments.

C. Single infections

The results of the present experiment prompted the study of the situation of changes in larval population with a single infection of 1000 eggs. Usually not all the eggs given in an infection have their larvae developing to adult worms. It was interesting to study the sequence of changes which takes place in the larval populations before the adult stage is reached. An experiment was planned but not to the same details as above. Chickens were given an infection of 1000 eggs at the age of two weeks. Two chickens were killed every week and total larval counts were done. The average weekly worm populations were as follows: 867 larvae in the first week, 500 larvae in the second week,

and 133 larvae in the third week. In the fourth week there occurred an average of 33.3 larvae and 5.2 adults per chick. These results were interesting in that following a single infection of 1000 eggs, there existed a marked variability of developmental rates within the larval population since even at the fourth week we find that while some individuals have completed their development to the adult stage many others were still held at the third larval stage, thus giving rise to the existence of two distinct populations. This shows some similarity to the finding of Madsen (1962) who mentioned that the population of worms was bimodally distributed as regards their size especially in heavy-dose experiments. This situation has also been noticed in other nematode infections, for example, with Ostertagia species in sheep Dunsmore (1960) also found two size modes in heavy-dose experiments. It is suggested that this pattern of development reflects an adaptive mechanism inherent in the host for combating the presence of parasitic infections. This will be discussed in more detail later.

SECTION 3

SEROLOGY OF INFECTION

Some information has been brought forward in sections 1 and 2 on the metamorphosis of the various instars and the dynamics of parasite population establishment under different conditions. Under conditions of high daily repeated challenge we have found consistent evidence of an arrest of development at the third larval stage. Even with single challenge infection of 1000 embryonated eggs there is a marked variability of developmental rates within the population since at the fourth week we find that whilst some individuals have completed their development to adult stage many others are retarded at the third larval stage. Delays in development are much less apparent at low infection levels. A priori we can argue that retarded development is a density-dependent phenomenon or alternatively that it is the result of a defence response by the host which increases in vigour with increasing challenge. Nevertheless, it appears that whatever the origin of the conditions adverse to development and survival the worms cannot counteract them except in the third larval stage. It is this stage only which shows any tendency to persist unchanged. At the same time it is to be noted that when conditions for inhibited metamorphosis of worms occur the chickens often show adverse

symptoms like loss of weight and retarded growth. On the other hand, chickens fed a low repeated infection of 10 eggs a day show a normal rate of growth and also a normal growth of the worms to adult egg laying populations. It is difficult to assess these two phenomena without a measure of immune response, if any, brought into play by the chickens as a result of these infections.

An antibody basis of immunity to Ascaridia galli was postulated as early as 1932 by Graham et al. They suggested that resistance of the host to A. galli infection was due to antibody formation during the period when larvae were in the mucosa. This view was supported by Ackert (1942). Although this opinion has prevailed since then, it has not hitherto been backed by a demonstration of the antibody in the serum of infected chickens. Sadun (1947 & 1949) was unable to demonstrate antibody by conventional methods using the precipitin test, although he showed an in vitro precipitate formed around the natural openings of larvae immersed in sera from infected chickens. In the present work attempts have been made to see whether any antibody can be demonstrated from chickens following repeated infections. Two tests were done: an agar-diffusion test - Ouchterlony plates, and a tannic acid haemagglutination test.

Boyden (1951) first described the conjugation of various proteins to tannic acid-treated erythrocytes, and the agglutination of these protein-conjugated red

cells by their corresponding anti-protein sera. The treatment of red cells with tannic acid brings about a change in their properties thus rendering them capable of absorbing proteins. The precise chemical changes in the cell surface responsible for this phenomenon are not known. It has long been known that tannic acid causes agglutination of red cells. Reinder and Fischer (1929) postulated that this is due to a change of the surface molecules of the erythrocytes from a highly hydrophilic to a hydrophobic state. Freund (1931) assumed that tannic acid produced a change in the surface potential of the cells so that agglutination occurred in the presence of certain electrolytes. Brading (1956), however, showed that tannic acid actually combines with the red cell surface. Whatever the exact mechanism be, it is not unlikely that the same factors are involved in the ability of the tanned cells to absorb protein so that agglutination follows in the presence of specific anti-protein sera.

The outstanding advantage of this reaction lies in its high sensitivity, its ease of performance and wide applicability coupled with the fact that the test requires only small quantities of reagents. Since the introduction of this technique it has been employed for the study of a large number of antibody-antigen systems derived from a wide variety of sources. In the field of parasitology it has been used quite extensively,

including, for example, the work of Kagan and Bargai (1956) for studies on the serology of trichinosis in rabbits, Jacobs et al. (1957) for the diagnosis and antibody assay of toxoplasma infections, and Soulsby (1956, 1957 & 1960) for the studies of antibody levels of nematode infested sheep.

Because of the sensitivity of this test it was decided to employ it for studying the antibody response of chickens infected with varying dosages of A. galli. Sera studied include those taken from experiment 3, where the population dynamics were studied of infection in chickens on a low protein diet with or without vitamin supplements, experiment 4 where population dynamics were studied of infection in chickens on 12.5 per cent and 15 per cent protein diets. Also weekly bleeding from chickens slaughtered for differential larval counts in section 2 (expt. 6) was followed up to study the trend of antibody response in a population of birds over a period of 19 weeks. Finally studies were carried out over a period of 4 weeks on chickens given a single infection of 1000 eggs. Before these tests were done on infected chickens, preliminary tests were done with bovine serum albumin (B.S.A.) to evaluate the technique of this reaction under a system containing chicken serum.

Material and Methods

Close attention was paid to glassware used for serological work. All containers were washed in acid-dichromate and dried in an oven. Bleeding was done by heart puncture and the blood run into bottles of appropriate sizes. Sera were collected from the clot after 24 hours and were stored in a deep freeze at -81°C.

Immunization of chickens

Serological tests were attempted first with B.S.A. Chickens used for immunization were six weeks old, and 50 mg of B.S.A. ~~of~~ in saline were injected according to the following schedule:

Day 1 50 mg subcutaneously
Day 2 50 mg intramuscularly (pectoral muscle)
Day 7 50 mg intramuscularly (do.)

Chickens were then bled on the fourteenth day.

Agar diffusion tests

The agar used throughout for diffusion tests was oxoid ion agar No. 2 (Code No. L. 12). Fowl antisera have been shown by Hektoen as early as 1918 to be atypical in their precipitin reactions. He noticed increasing specifity with increasing salt concentration. This phenomenon has been further examined since then and it is now generally agreed by Goodman, Wolfe and Norton

(1951), Makinodan et al. (1960) and Orleans et al. (1961) that increased precipitation occurs with increase of salt concentration from 0.85 per cent to 8 per cent. This effect is not a salting-out, as it is only possible to have a salting-out effect when the salt concentration is of the order of 18 per cent. Bearing these findings in mind, the agar used was made up in phosphate-buffered saline at pH 7.2 at a concentration of 0.5 per cent. No filtration was necessary. The salt concentration used was 8 per cent, and merthiolate was added for preservation at a concentration of 1 in 10,000. No dilution of test sera was done, and the tests were carried out on Ouchterlony plates.

Techniques for worm antigen preparation

Previous workers like Oliver-Gonzalez (1946), Thorson (1951), Chandler (1953) and Soulsby et al. (1959) have emphasised the importance of metabolic products of worms as the 'functional' antigen which induces the formation of antibodies against parasitic infections. This means that during studies involving the assay of antibody production on helminth infestations the antigenic extractions most likely to give positive results would be those prepared in such a way as to obtain in a non-denatured state the maximum possible concentration of the metabolic products of the worms to be studied. Bearing this in mind, an attempt was made

to obtain 'functional' antigens during the preparation of antigen for this work, and to preserve as far as possible their natural character as obtained from the living worms. This entailed preparation in the cold as much as possible.

Antigen extraction

Larvae and adults were processed in the same way. The frozen weight of the worms was determined, and they were transferred from -81°C to +4°C to thaw for about 2 hours after which they were transferred to the ice chest of a refrigerator for 24 hours (temperature -5°C). This was done four times. The alternate freezing and thawing was to form crystals inside the cells of the worms to cause their breaking up to release their protein materials. The worms were thoroughly homogenized using a TRI-R Teflon tissue homogenizer (Camlab Ltd., Cambridge). This equipment consisted of a variable speed laboratory motor instrument with a stainless steel shaft carrying a 15 per cent glass reinforced teflon pestle. This material can withstand high temperatures, is resilient and has a smooth, waxy non-wettable surface. These properties facilitate sterilization, reduce the heat generated, eliminate contamination from glass particles and preserve the controlled clearance between pestle and tube. The pestle could be rotated over a wide range of speeds. The tube was a pyrex precision bore homogenizing

glass tube, which was immersed in ice during homogenization of the worms to offset the rise in temperature due to the revolving pestle. Extraction was done with saline at the rate of 4 ml per gram of original worm material, allowed to stand at 4°C for about two hours and then centrifuged in the cold for 20 minutes at 3000 r.p.m. The particle-free opalescent supernatant fluid was collected and stored at -81°C in 4 ml aliquots in bijou bottles.

Both larval and adult worms were used for antigen preparation. The larvae were collected from chickens previously fed about 5000 infective eggs at the age of two weeks and maintained on a diet of low protein (10 per cent). After some three weeks, the chickens were slaughtered, their intestines removed and slit open the whole length and subjected to partial digestion. The digesting fluid consisted of 2 per cent w/v pepsin solution in 0.7 per cent hydrochloric acid v/v. The whole was incubated at 37°C for about two hours. After this several larvae were free from the mucosa and found swimming around in the digesting medium. These larvae were harvested by a modified Baerman apparatus over a hot plate. The apparatus was assembled by tying a gauze cloth of uniform porosity over the mouth of a jam-jar containing the partially digested intestines. This was carefully inverted over a petri-dish. A little normal saline was added to the dish and the whole placed

on a hot plate at 40°C. There was a tendency for live larvae to migrate through the cloth into the petri-dish where the temperature was higher, and they swam around in the saline. They were pipetted out into a measuring cylinder and further cleaned out by shaking up several times with warm saline. The debris sank to the bottom of the cylinder while most of the larvae remained suspended and were decanted. This procedure could be repeated as many times as was necessary and finally the larvae concentrated by centrifugation at low speed. Further purification when necessary was done by hand-picking. The larvae harvested were gently dried in filter paper and then frozen at -81°C and then kept at this temperature until required.

Adults for antigen preparation were used while still young. Chickens infected as above were killed after about five weeks, and adult male and female worms were collected by flushing out the intestines with warm water, and placing them immediately in warm normal saline at 37°C for washing. After this, they were dried as before and stored at -81°C as stock, until enough material had been collected.

Preparation of erythrocytes for haemagglutination test

Two types of red blood cells were used for these tests, namely, sheep and poultry. Blood from sheep was obtained from the Edinburgh abattoir. Poultry blood

was received from a poulterer in Edinburgh. In each case the blood was run straight into 1.2 volumes of modified Alsevers solution with gentle swirling.

The original haemagglutination technique of Boyden (1951) employed the use of fresh cells. This technique, though sensitive, has the disadvantage that fresh cells have to be collected frequently, fresh sensitized cells have to be prepared for each day's titration of sera, hence reproducibility of results is limited and effective comparison of different experiments is difficult. As blood cells age, their surface characteristics diminish, and non-reproducibility increases, possibly due to alterations during their in vitro storage, largely by bacterial action. Apart from this there is the added disadvantage of lysis which occurs readily in fresh cells. Cells were found to lyse very easily due to changes in pH, by mechanical damage during centrifugation and through accidental changes in tonicity. These factors make the use of fresh cells for haemagglutination very undesirable.

For these reasons, attempts have been made by several workers to prepare erythrocytes which can be stabilized and used for a long time with retention of surface properties for agglutination tests, thus permitting better reproducibility in tests carried out at different times.

Flick (1948) was the first person to note that red

cells treated with formaldehyde could be preserved for long periods of time. He found that human erythrocytes so treated retained their ability to react with influenza virus in a manner quite similar to that of untreated human erythrocytes. Following this, several investigators, notably Cole and Farrell (1955), McKenna (1957) and Ingraham (1958) devised various methods of preparing formolized red-cell reagents for subsequent work in which special characters of the cells were to be utilized. Many of the early methods were non-reproducible, and often showed spontaneous agglutination with a trace of formaldehyde.

One of the best methods, by which large amounts of red cells can be formalinized and stored ready for use, is that of Csizmas (1960). The method used in the present work was based on Czismas' method with slight modifications to suit available equipment.

Blood cells were collected as above and were processed without delay so as to utilize the cells in their freshest state. When, however, blood was not to be used immediately, the Alsevers solution had to be autoclaved before the blood was run in, and after that kept cool at $4^{o}C$ until required. Before formalinization, the blood was gently mixed and the P.C.V. determined as described previously, and then made up in cold normal saline in a plastic bucket to form a suspension of approximately 5 per cent. This blood suspension was

gently mixed with a glass rod, avoiding foaming as much as possible, and the cells recovered and packed through a chemical centrifuge. This was the first wash. The procedure was repeated twice more. This method of using a chemical centrifuge for washing the cells was greatly superior to the use of a bucket centrifuge on account of the quantity of cells processed, the amount of time and labour saved and the thorough washing to which the cells were subjected with comparatively little destruction by foaming. This thorough washing was necessary before formaldehyde treatment to avoid the stickiness which can result from the gelling of plasma proteins on the cell surface.

After the last wash, the P.C.V. percentage was determined again and the cell suspension made up to 10 per cent using 0.15 M phosphate buffered saline of pH 6.9 for chicken cells and 0.85 per cent saline for sheep cells. Formalin (40 per cent formaldehyde) of one-quarter the volume of the cell suspension was poured into a cellophane dialysis sac twisted and knotted at one end. The tubing was cut off leaving a one-third length of the sac from which air was expelled and finally knotted off. This sac was placed at the bottom of a beaker and the cell suspension poured over it. The beaker was placed on a light metal grid carried placed on an inclined turn-table, set to allow slow revolution, just enough to keep the cells suspended with

minimum foaming. When this was not available similar results were produced by placing the beaker on a magnetic stirrer and suspending a rod magnet dipping into the cell suspension from a fisherman's spinner held on a retort stand. This gentle agitation was carried out for four hours after which the sac was punctured, releasing the rest of the formaldehyde into the cell suspension. Stirring was continued overnight. In the morning, the reddish-brown suspension of cells was decanted slowly into a clean container taking care to exclude the floating mass of broken cells and debris. Next, the cells were passed through a 100 mesh sieve to remove any clotted blood cell particles, thus leaving a cell suspension free of clumps and other debris. These cells were then washed again in a 5 per cent suspension and sedimented in a chemical centrifuge as previously described. After the first wash, the packed cells were resuspended to form a suspension of 25 per cent, and stored at $4^{\circ}C$ in well corked medical flasks as stock suspension.

This method of preparation produced a well dispersed suspension of cells morphologically undistorted by the formalin treatment. This was because of the pH at which this was carried out. At a low pH of 6.9 the cells were swollen up and rounded, and little crenation occurred. This was good for the formation of good settling patterns during the haemagglutination test. The suspension of

cells could not be lysed by distilled water, not even in the presence of complement and specific antibody, and was very resistant to mechanical damage even to freezing at -81°C for months. The stock suspension was used for over one year without any noticeable changes in property or reaction. The agglutinations were reproducible using as control a standard test serum (chicken anti-B.S.A.). Plate 1 shows sheep and poultry cells prepared in the above manner.

The technique for haemagglutination tests

Requirements

1. Formalinized sheep or poultry red cells prepared as above.
2. McCartney bottles or Universal containers graduated prior to the test to show 10 ml, 20 ml and 25 ml levels.
3. Phosphate buffered saline (hence called P.B.S.) made up as follows:

   3.407 g per litre 0.01 M di-sodium hydrogen phosphate $Na_2HPO_4 \cdot 12H_2O$; and

   1.294 g per litre 0.01 M potassium dihydrogen phosphate KH_2PO_4.

   These were mixed in the ratio of 7:3 to give a buffer of 0.01 molar concentration. Before use a one to one mixture of the phosphate buffer and 0.284 M sodium (i.e. 1.642 per cent NaCl)

chloride was made. This makes 0.01 M P.B.S., whole total molarity being 0.147 M, i.e. the molarity of normal saline.

4. Tannic acid (analar). A 1:10,000 solution was used for tanning red cells. This was made up by dissolving 2.5 mg in 25 ml of P.B.S.
5. Antigen for coating cells (20 mg in 10 ml of P.B.S. in case of B.S.A.).
6. Perspex agglutination plates.
7. Normal rabbit serum or normal guinea-pig serum (absorbed).
8. Worm antigen 6 ml for each 25 ml of 1 per cent cell suspension.

Procedure for test

This procedure reported here was found to be the most successful and reproducible after trials had been carried out. The tests leading to the establishment of this procedure will be described briefly at the end.

1. 1.1 ml aliquot of cell suspension from the 25 per cent stock suspension was measured into each of the McCartney bottles. The extra 0.1 ml of cell suspension allowed for losses during the numerous washes to follow. One bottle was for the preparation of the cells to be used as controls and the other was for cells to be coated with antigen. These two vials were made up to 10 ml mark with P.B.S. and spun down and the supernatant poured off.

2. Treatment with tannic acid

Cells in each bottle were re-suspended and made up to the 10 ml mark with P.B.S. and tannic acid solution was added to each bottle to the 20 ml mark. The cells were mixed by swirling, and then stood for 15 minutes in a water bath at 37°C. After this, the tanned red cells were spun down at 400 g for 3 minutes and the supernatant sucked off. The cells were then re-suspended in P.B.S. up to the 20 ml mark to wash once, and spun down again. It was noticed that it was much easier to re-suspend packed cells by swirling with 1 to 2 ml of P.B.S., the rest being added when re-suspension had been obtained. This minimized foaming and destruction of cells.

3. Adsorption of antigen by the red cells

Putting one bottle aside, the red cells in the other bottles were re-suspended in 10 ml of P.B.S., and then 10 ml of antigen solution were added. The mixture was swirled round carefully to avoid antigen destruction by foaming and then incubated at 37°C in the water bath for 15 minutes, swirling the bottle every 5 minutes. After 15 minutes the cells were spun down and the supernatant removed.

4. Stabilization wash in normal rabbit serum (N.R.S.)

The cells in both bottles, one containing red blood cells coated with antigen and the other containing tanned cells only, were now re-suspended in 20 ml of P.B.S. containing 1 per cent normal rabbit serum and spun down

to give a single wash. They were then finally re-suspended and made up to 25 ml with 1 per cent normal rabbit serum in P.B.S. This gave approximately 1 per cent cell suspension ready for use for the test.

5. The test

The test sera were first absorbed to remove natural antibodies to the cell type being used for haemagglutination. This was achieved by adding 0.9 ml of 20 per cent red blood cells suspension in P.B.S. to 0.1 cc of test serum using an automatic pipette. Normally at bench temperature only 10 minutes absorption was found necessary, after which the cells were spun down leaving an optically clear dilution of test serum free of natural antibodies to the test cells. Using a 0.1 ml capacity automatic syringe serial doubling dilutions of test sera were made in the perspex agglutination trays using 0.1 ml of 1 per cent normal rabbit serum as the diluent. The last well of each test series was left as a control against non-specific agglutination of test cells. To this control well was added a 1:10 dilution of test serum, and 0.1 ml of uncoated red cells. To the remaining wells were added 0.1 ml of coated red cells. Two saline controls were also included, one for antigen-coated cells, the other for uncoated cells. Reading was done after about 12 hours, although the settling pattern of the cells could already be discerned after one hour. Agglutination was taken to occur in those

wells where the cells had failed to form a button.

Tests for the evaluation of technique

1. Natural antibodies to red blood cells

The first test was to note the extent of natural antibodies to poultry red cells (P.R.C.) and sheep red cells (S.R.C.) in normal rabbit serum. The test serum (normal rabbit serum) was diluted out in doubling dilutions (0.1 ml aliquots) and then 0.1 ml aliquots of 1 per cent P.R.C. suspended in P.B.S. were delivered to all the wells. This was repeated for S.R.C. Iso-agglutination of P.R.C. was observed down to a dilution of 1:320 of rabbit serum, and of S.R.C. to a dilution of 1:40 of rabbit serum. This showed that treatment of cells with formaldehyde did not destroy the property of the red cells to exhibit isophile reactions such as iso-agglutination in the presence of natural antibodies against them. It was also necessary to absorb normal rabbit serum before its being used for the test. Absorption with a 20 per cent cell suspension of P.R.C. or S.R.C., according to the case, was sufficient to prevent iso-agglutinations. This was also the case with normal chicken serum, and normal guinea-pig serum when they were used as diluents for the test.

2. Titration of various sera for stabilization of cells

P.R.C. as well as S.R.C. after treatment with

tannic acid were seen to auto-agglutinate in the P.B.S. Several materials, such as normal rabbit serum, 0.2 per cent solution of gelatine, bovine gamma-globulin, etc., have been used by various workers as diluents for stabilizing cells. Attempts were made here to get a stable material for stabilizing these cells to prevent auto-agglutination during the test. The sera tested were normal chicken serum, normal rabbit serum and normal guinea-pig serum. Serial dilutions of the absorbed sera were done as described, and 0.1 ml aliquots of 1 per cent test cells run into each well and mixed up by gentle agitation. Stabilization patterns were read later. Stabilization was taken to occur in those wells where cells settled down to form a perfect button. Contrary to what might be expected, P.R.C. could not be stabilized in any dilution of normal chicken serum, even after absorbing the serum. They could, however, be stabilized in normal rabbit serum of dilutions 1:10 to 1:320 and the optimal titre for stabilization lay between 1:80 and 1:160. This was exactly the same when using guinea-pig serum although the stabilization pattern with this was not very good.

S.R.C. also showed best stabilization in normal rabbit serum, the optimal titre also lying between 1:80 and 1:160. This was followed by normal guinea-pig serum and finally normal chicken serum although stabilization titres in the latter ran from 1:10 to 1:80. From this

test, it was found that about 1:100 normal rabbit serum was very good for stabilization in either sheep or poultry cells and also for diluting the test sera. Sheep red cells were ultimately used throughout the tests because they produced the best stabilizations.

Results

A. Preliminary studies with B.S.A. to evaluate technique

The chicken anti-B.S.A. sera obtained from immunized chickens had formed good precipitation lines during immune diffusion studies. This is shown in Plate 2. Nevertheless, when these antigen and antibody were used for the haemagglutination test, there was no agglutination. No reason could be found why this crystalline B.S.A. from Lights and Co. could not be adsorbed on to sheep or poultry cells for the agglutination test. New sets of chickens were, therefore, immunized against normal bovine serum obtained from the Burroughs-Welcome laboratories. The schedule for the administration of this antigen was exactly the same as that used previously. Using 0.3 ml of bovine serum for coating the red cells, the agglutination test worked and the titres from the two immunized chickens were 1/5120 and 1/2560 respectively. This titre was reproduced on all other occasions when trials were carried out to see if there existed a change in the titre of immune sera on storage. Plate 3 shows agglutination titres of the

TABLE 14

ANTIBODY TITRES WITH INFECTION
(PRELIMINARY TRIALS)

A					B			
DIET	Grade of Infect.	Chicken	Titre at 4 wks.	Titre at 8 wks.	DIET	Grade of Infect.	Chicken	Titre at 8 wks.
10% Pr. with Vit. Suppl.	R.1.10	475	-	40	15% Pr.	R.1.10	340	20
		477	-	20			341	40
		479	-	20			344	-
"	R.1.100	480	-	40	"	R.1.100	346	80
		481	-	80			348	80
		485	-	20			350	40
"	R.1. 1000	486	80	80	"	R.1.1000	352	40
		488	160	80			353	160
		491	80	80			356	160
10% Pr. No Vit. Suppl.	R.1.10	498	-	-	12.5% Prot.	R.1.10	364	20
		499	-	-			366	20
		500	-	-			368	20
"	R.1.100	318	-	-	"	R.1.100	370	20
"		319	-	-			374	40
		320	-	-			375	20
"	R.1.1000	322	20	-	"	R.1.1000	376	80
		324	-	-			378	80
		326	-	-			380	40

sera after storage from two chickens immunized against bovine serum.

B. Chickens infected with A. galli

The highest titres at a single infection of 1000 eggs was 1/40 in the second week, and 1/20 in the third and fourth weeks. Antibody titres of sera from chickens under daily repeated infections of 10, 100 and 1000 eggs per day both from the experiments carried out on low protein (10 per cent) with or without vitamin supplements and the experiments carried out on 12.5 per cent and 15 per cent protein are shown in Table 14. Titres obtained from chickens under low protein nutrition were very low. At four weeks of infection, only chickens under repeated daily infection of 1000 eggs and on a vitamin-supplemented diet had any antibody titres, the highest being 1/160. Only one chicken under daily repeated infection of 1000 eggs on a diet lacking vitamin supplements had a titre and this was as low as 1/20. In a second trial at eight weeks, all groups of chickens on the vitamin-supplemented diet showed titres. Again the highest titres were shown mostly by chickens under daily repeated infection of 1000 eggs. No chickens on a diet without vitamin supplements showed any titre.

Chickens on higher protein diets (12.5 per cent and 15 per cent) showed titres in the eighth week of infection (see Table 14). The highest titres from

TABLE 15 : EXPERIMENT 6

ANTIBODY TITRES OF CHICKS BEFORE SLAUGHTER AT WEEKLY INTERVALS FOLLOWING INFECTION WITH 10 AND 1,000 EGGS PER DAY

Groups	Wk. 1	2	3	4	5	6	7	8	10	12	16	19
R.1.1000	0	2,560	820	160	160	40	80	80	40	320	0	20
	20	2,560	160	160	160	80	40	40	80	40	40	20
	0	1,280	160	160	320	160	160	160	80	80	20	20
R.1.10	0	0	20	40	80	40	20	80	20	0	0	0
	0	0	20	40	40	80	40	20	40	20	0	0
	0	0	40	20	40	80	0	40	80	20	0	0

R.1. 1000 = Repeated Infection 1,000 eggs
R.1. 10 = " " 10 "

either dietary type came from chickens under a repeated infection of 1000 eggs, the highest being 1/160. The lowest titres were from groups fed on a daily repeated infection of 10 eggs.

Following the development on a diet of 15 per cent of antibody titres of chickens under repeated infection of 1000 and 10 eggs respectively for a period of 19 weeks (Table 15) & Figs. op. p. 75. it was again noticed that the highest titres throughout this study were obtained from chickens under a daily repeated infection of 1000 eggs. In this group also the highest titres were obtained in the second week of infection. During this period, however, hardly any titres were obtained from chickens under a daily repeated infection of 10 eggs.

Failures to demonstrate antibody from infected chickens by earlier workers have been mentioned already. During the present work attempts to get precipitation lines by immune diffusion tests in agar failed using chickens under varying dosages of single and repeated infection with A. galli. Even after removal of lipoid materials from the antigen extract, precipitation by agar diffusion was not obtained. Using the haemagglutination test, however, titres were obtained as shown in the results. The failure to get a positive agar-gel diffusion test could be due either to a failure of chickens infected with A. galli to produce precipitating antibodies or to the precipitating antibodies produced

being in too low a concentration to be measured by the precipitation test. Although the apparent ability of the haemagglutination test to detect so-called non-precipitating antibodies was reported by Borduas and Grabar (1953), there is no known record of nematode infections being able to produce such antibodies. Also, recent work by Fiset (1962) has shown conclusively that the haemagglutination test is not capable of measuring incomplete antibodies. From these considerations one conclusion only can be sustained to explain the failure of agar-diffusion tests to identify antibodies to Ascaridia galli in chicken sera which give positive haemagglutination. This is that the precipitating antibodies are in insufficient concentration for identification by the less sensitive gel-diffusion test whereas the more sensitive haemagglutination test is able to detect them.

The haemagglutination titres obtained tend to suggest that much of the antibody produced is against the larval population. Under a low daily infection of 10 eggs, few larvae occur, and the immune response is very low in comparison to the situation obtained when chickens are given a daily larval dosage of 1000 eggs. Also, the highest titres under a repeated infection of 1000 eggs occurred in the second week, when the highest larval population was obtained in the group. That a high adult population can also elicit some response is

shown by the fact that a moderately high titre was shown at the sixth week, when a good adult population was present, by the chickens receiving a daily infection of 10 eggs. Nevertheless, the best responses were usually obtained when the larval population was high. Host nutrition also plays an important part. This is shown by the chickens from experiment 3 which were fed on a low protein diet without vitamin supplements. In them, even when high larval or adult populations were present, the antibody titres were minimal or nil.

SECTION 4

BLOOD PROTEIN STUDIES AND PATHOLOGY OF INFECTION

This section is devoted to blood protein studies, and to a general discussion on the pathological effects of infection. Previous experiments on the growth of infected chickens gave figures which showed that significant weight changes occurred with infection, especially in chickens under high production nutrition. In them differences in growth rates as measured by weight gains could be shown more clearly. Several reasons could be suggested for these weight differences:

a. Infected chickens may have failed to eat, i.e. loss of appetite.

b. Worms might be competing with the chickens for ingested food.

This has been shown by Ackert et al. (1940) who found that the parasite depended chiefly on the host ingesta, and also by Reid (1944) who found that worms were eliminated with starvation of the host.

c. The parasites could be releasing toxic substances which might affect the liver and hence the production of proteins.

d. There might occur a cumulative loss of blood due to continual penetration of larvae into the mucosa during the course of repeated worm challenge.

If a. or b. be the case, starvation must occur, and this might be reflected in an overall depletion of the protein reserves in the serum. If, however, c. or d. be the case, we should expect changes in the albumin concentration of the serum. To explore these possibilities further, total protein and albumin fractions were determined from sera obtained from chickens used in experiment 4.

Material and Methods

The technique for Biuret reagent determination of proteins used here was that of Weichselbaum (1946).

Several Biuret techniques have been developed, all based on the reaction of freshly precipitated copper oxide or an appropriate copper salt dissolved by an alkaline protein solution to give a Biuret reaction: a violet colour, proportioned to the amount of protein present. This colour is relatively stable and applicable to protein fractions. In practice the addition of copper sulphate solution to an alkaline solution is convenient. In most cases, however, the Biuret colour is unstable and turbidity often occurs to mask the reading.

The Biuret solution of Weichselbaum is optically clear, relatively permanent in colour and, when added to protein dilutions, forms a relatively permanent optically clear Biuret complex which obeys the Beer-Lambert law.

To obtain this stable complex, sodium potassium tartrate is used to react with the copper salt and the pH optimum for this is that of approximately 0.2 N sodium hydroxide. The Biuret complex remained optically clear and stable for at least five days.

The added potassium iodide is to prevent the auto-reduction of copper tartrate solution on standing.

Procedure

1. 0.5 ml of unhaemolysed serum was transferred to a graduated centrifuge tube and 23 per cent sodium sulphate solution was added to the 10 ml mark. This was mixed well by repeated inversion, care being taken to avoid excessive foaming. A 2 ml aliquot of the uniform protein suspension was immediately pipetted into a separate small test tube (A) for total protein determination. To the remainder was added 3 ml of ethyl ether. The test tube was shaken properly and centrifuged for about 5 minutes. After this, the tube was held in a slanting position and the tip of a narrow bore pipette inserted past the packed globulin precipitate lying in the ether layer, into the clear aqueous layer below. 2 ml of this was sucked up and transferred into a second test tube (B) after wiping off any adhering precipitate. This tube (B) is for albumin determination.

2. 5 ml of Biuret reagent were measured into the two tubes, mixed well and incubated in a water bath at

31°C for 30 minutes.

3. The control serum used was one of the Versatol series, a freeze-dried, pooled human serum containing serum proteins of standardized values. Of the available range, Versatol-A Alternate was used throughout this experiment because the protein value was of the range 4.5 g total protein per 100 ml of blood which although relatively abnormal for human blood is closest to the range occurring in poultry. This was obtained from the General Diagnostics Department, William R. Warner and Co. Ltd., Hampshire. The tubes of dried serum were reconstituted with 5 ml of distilled water each, and left standing for 30 minutes before use. 0.5 aliquots were used as standard controls in the Biuret test.

4. Reference blanks consisting of 2 ml of 23 per cent sodium sulphate and 5 ml of Biuret reagent without protein were also incubated as in the test.

5. Colorimetric determination was carried out with a green filter (Chance OGR 1).

6. On each occasion determinations were made of total protein and albumin fractions.

Results

The results are shown in Table 16.

The occurrence of sporadic diarrhoea complicated the interpretation of results obtained from blood work as we have shown in experiment 1. Also, the result of protein

TABLE 16

BLOOD PROTEIN LEVELS 8th WEEK AFTER INFECTION

DIET	15% PROTEIN				12.5% PROTEIN			
Grade of Infect.	Total Prt.	Albumen	Globulin	A/G	Total Prt.	Albumin	Globulin	A/G
R.1.10	3.2	1.57	1.63	0.96	2.5	1.22	1.28	0.95
	2.7	1.20	1.50	0.80	3.2	1.54	1.66	0.93
	3.1	1.54	1.56	0.99	2.4	1.16	1.24	0.94
R.1.100	3.4	1.67	1.73	0.97	2.8	1.33	1.47	0.90
	3.1	1.45	1.65	0.88	3.0	1.43	1.57	0.91
	3.2	1.46	1.74	0.84	3.2	1.55	1.65	0.94
R.1.1000	3.0	1.45	1.55	0.94	3.4	1.62	1.78	0.91
	3.8	1.75	2.05	0.85	3.6	1.62	1.98	0.88
	3.7	1.66	2.04	0.81	3.2	1.42	1.78	0.80
Control	3.0	1.47	1.53	0.96	3.1	1.52	1.58	0.96
	3.4	1.63	1.77	0.92	2.9	1.41	1.49	0.95
	2.9	1.41	1.49	0.95	3.0	1.43	1.57	0.91

R.1 10 = Repeated infection 10 eggs a day
R.1 100 = " " 100 " " "
R.1 1000 = " " 1000 " " "

depletion in animals is often accompanied by a reduction of plasma volume (Alison, 1953). The concentrating effect of loss of plasma fluid can often be so marked that an estimate of blood proteins without reference to the plasma volume becomes meaningless. In such circumstances the comparison of serum protein levels in terms of concentration of total protein leads to serious difficulty through relative changes of the different protein components. Alison (1955) shows that this can be partly overcome by expressing shifts in plasma proteins as albumin-globulin (A/G) ratios. On this account, albumin-globulin ratios were studied and subjected to a statistical analysis while the absolute values of albumin and globulin and their sums (total protein) were ignored. There were no significant differences in the A/G levels either between birds on the two diets (15 per cent protein and 12.5 per cent protein levels) or between the groups on different levels of infective challenge. From these results, we can conclude that there are no significant effects on the blood protein levels of chickens on a diet with 15 per cent or 12.5 per cent protein due to worm-infections resulting from continuous challenge at any of the levels studied.

Pathology and Pathogenicity of Infection

Failure of infected chickens to gain as much weight as the controls has already been stated. Infection

with A. galli especially at high levels is frequently accompanied by a serious diarrhoea which lasts for up to five weeks after which symptoms become intermittent and sporadic. This is most severe when the chickens are living on a low protein diet. The peak of pathogenic effects under these circumstances of repeated infection occurs mainly in the third week, and when the protein diet is low mortality may be high from this period on. This is accompanied by loss of appetite and the birds show a decreased activity with ruffled plumage, drooping wings, and a hunched-up drowsy appearance. Growth is arrested at this stage as a consequence, in part at least, of the behaviour of the affected chicken. Evidence of intestinal damage is found in the passage of blood-stained diarrhoeic stools. Most chickens become progressively emaciated and feed sparsely while showing little interest. They develop conspicuous leg weakness and eventually remain prostrated until death supervenes. These symptoms may last for up to one week before the chicken eventually dies. As would be expected, the most severely affected chickens are those on the least nutritious diet and sustaining the highest levels of repeated worm-challenge. With a high protein diet most chickens recover after a short period and some may then develop a voracious appetite. When chickens affected as above are housed together much of the mortality which occurs is directly due to cannibalism. It was noticed

that chickens showing signs of weakness or decreased activity were instantly pecked to death, hence the need to house individual chickens in separate compartments which became apparent during the course of this project.

Lesions

Macroscopic lesions were frequently found associated with infection. They were readily visible to the naked eye as haemorrhagic patches intermixed with mucus and larvae (Plate 4). These are very prominent in the duodenum of infected chickens autopsied in the third week, and may continue for a longer time (up to the eleventh week) in chickens on a low protein diet and receiving a daily repeated infection of 1000 eggs. Strings of blood clots are often seen on their way to be passed out in the faeces (Plate 4). Scar tissues are left on the intestinal epithelium of chickens which survive (recover) and they assume a semi-whitish appearance which can be mistaken for foci of coccidial infection. In chronic infections, there occurs a loss of muscle tone and the intestinal walls assume a flabby appearance. Such intestines usually contain large numbers of adult worms and larvae. In severe cases the body musculature is also affected and the birds become emaciated. This is especially evident in the breast muscle (Plate 5). Such chickens hardly ever recover.

Histological examination of infected epithelium

often reveals extreme destruction and erosion of the glandular epithelium by migrating larvae (Plate 6). Associated with the presence of larvae there is a proliferation of mucus-secreting cells which often results in adhesion of the villi. Numerous vascular extravasations occur in the villi, giving them a distended appearance. Deep migration of larvae is rare. They are seen mainly in the mucus membranes between, or occasionally within, the villi. A few may penetrate as deeply as the muscular layers (Plate 7), and occasionally some migrate into the bile or pancreatic ducts (Plate 7). No larvae were seen in the lungs, kidney or liver. Not all the larvae that penetrate the epithelium survive, for some are arrested and surrounded by fibroblasts (Plate 8).

The phenomenon of cellular infiltration due to infection is difficult to judge in the avian subject. Chickens are known to have well developed lymphoid areas (Peyer's patches) in the intestine. Occasionally hyperplasia of these lymphatic nodules occurs both in infected chickens and in the controls. One cannot therefore relate this phenomenon exclusively to infection (Plate 9), more particularly since the lymphocytes are not arranged in any sequence around the worms seen in the tissues. There is virtually no eosinophilic response to be observed in this system. Fibroblasts occur only in relation to dead larvae.

Damage to the chickens and to the epithelia of the intestine is not due solely to larval worms. Adults, when present in large numbers, migrate up and down the intestinal lumen. They have been seen in the gizzard, where they may penetrate slightly into the epithelium through breaks in the cornified layer. Adult worms may migrate occasionally into the caecum of infected chickens (Plate 10). The greatest harm caused by them, however, is through obstruction of the intestine (Plate 11). † (Plate 12.) This phenomenon normally occurs in the lower half of the intestine. It is not known what causes worms to aggregate and thus cause occlusion, but when it occurs the worms of the bundle are very closely applied to the mucosa of the intestine, thus leading to accumulation of fluid and obstruction of the food passage. They may remain in this position for weeks, leading to a slow death of the chickens. Histological examination of such areas shows extensive damage through atrophy of the villi from pressure and perhaps from toxins released by the worms. Necrotic changes often take place and the mucosa loses the appearance of an absorptive membrane. In some cases metaplastic changes may occur before death: the epithelium becomes cornified in places. Few chickens recover after this stage is reached and in most cases death supervenes from starvation. This phenomenon is not the rule in all cases of infection. It is seen most frequently in chickens on a low nutritive diet

while those on an adequate nutrition are able to recover before this stage of infection is reached. The recovery is often sudden, leading to an extrusion of a bundle of worms which can contain up to twenty adults including both sexes.

SECTION 5

In the course of the experiments described above two principal results have been described. First, the pattern of establishment and development is different for high and low rates of challenge. Secondly, the titre of antibody to helminth antigen is different following high and low rates of challenge. Low challenge in these experiments (10 per day) usually produced infestations in which a significant proportion of the worms developed to the adult stage and produced eggs. High challenge (1000 eggs per day) produced infestations in which the adult worms were significantly reduced in number and delayed in appearance. At the same time substantial numbers of worms persisted in the third larval stage. Anti-helminth antibody developed in highest concentration in the birds receiving 1000 eggs per day. We find, therefore, a correlation between the two sets of observations. Resistance to helminths, as evidenced by retarded and reduced adult development and by inhibition of development of third-stage larvae, developed in response to high challenge levels which also induced the development of humoral antibody. Low challenge provoked neither resistance nor antibody production. Moreover, severe malnutrition incapacitated both resistance and antibody responses even to high challenge.

It is pertinent to enquire whether the resistance phenomena are mediated by the circulating antibody or whether the two are independent manifestations of the defensive host-response. Alternatively, the question can be posed whether resistance to infection might occur in the absence of circulating antibody response. The present experiment was undertaken to explore these questions by inhibition of the antibody response.

Many agents are known to inhibit the genesis of antibody to immunogenic substances. This property has been utilized therapeutically in attempts to alleviate the course of auto-immune diseases and to suppress homograft responses. Antibody production can be inhibited by X-irradiation and by chemical agents. Irradiation has been quite extensively studied by Taliaferro et al. (1952), Dixon et al. (1952) and Berenbaum (1962) among others. Among chemical agents effectiveness has been established for nitrogen mustards (Spurr, 1947), corticosteroids (Germuth et al., 1950), 6-mercaptopurine (Schwartz et al., 1958), and, more recently, 6-thioguanin (Sterzl, 1961, and Berenbaum, 1962 & 1964). The action of these substances on the antibody response is not simply due to general toxicity. Many toxic substances are quite without demonstrable effect on antibody production even in lethal doses. Effective agents, on the other hand, may inhibit antibody synthesis markedly in doses considerably below the toxic range. The agents

used to inhibit antibody response in the present work were 6-thioguanine administration and X-irradiation.

6-Thioguanine, an active purine analogue, has been used extensively (Eisen et al., 1962, and Cancer Chemotherapy reports, 19). Its use as an inhibitor of antibody production is recent. Toxic effects from overdosing occur mainly in the bone marrow of mammals where it causes a reversible aplasia. It does not, however, produce direct damage to lymphoid tissue (Philip et al., 1956). The mode of action for the inhibition of antibody production is not yet known. Sartorelli and Le Page (1958) reported that it is an anti-metabolite which interferes with some phases of nucleic acid purine metabolism. It acts chiefly by the inhibition of the uptake and incorporation of guanine, thus leading to an equal depression of DNA and RNA. Whatever its mode of action may be, it is possible that it differentiates antibody-producing cells during the induction period following antigenic stimulus. Berenbaum (1962) found that the effective suppression of immune response by this drug depended on timing of the treatment. He obtained the strongest suppression by injecting the drug about two days after the antigen. He explained this action by the hypothesis that immunologically competent cells are derived from precursors that are themselves unresponsive to antigens. These precursors give rise by differentiation to cells able to

respond to antigen. If these cells receive an appropriate antigenic stimulus during this stage, they undergo a further rapid differentiation and it is at this induction period that they become susceptible to the effects of thioguanine. In the absence of the anti-metabolite, these cells become fully differentiated and produce antibody and are now insensitive to 6-thioguanine. When drug is administered after antibody production has started, or before the induction period, it is incapable of inhibiting antibody production. This theory emphasises the importance of correct timing in the administration of drug and antigen for the maximal depression of antibody production.

The importance of timing with respect to X-irradiation and the suppression of immune responses has been widely recognized and studied by, for example, Dixon et al. (1952), Taliaferro et al. (1952) and Berenbaum (1962). It is generally agreed that maximal suppression of anti-body response is obtained when irradiation is carried out from two to four days before administration of antigen. The mode of action is again incompletely understood. Moderate doses of X-rays destroy lymphocytes, but regeneration occurs within a week, thus suppression of antibody response cannot be attributed solely to destruction of lymphocytes. The loss of the ability to form antibodies by irradiation is often regarded as an aspect of the reduction of protein synthesis.

Inhibition of the synthesis of properdine, and euglobulin has been quoted by Bacq (1961) and this might be the main effect of irradiation. Berenbaum (1962) suggests that X-rays interfere with the development of antibody-producing cells from their precursors.

Methods

a) Irradiation of chickens

Chickens to be irradiated were held in a small gauze-cloth bag held to a square wooden board with drawing pins. This kept the chickens steady throughout the irradiation period. The chickens were treated singly, and no part of the body was sheltered from the X-rays. The instrument used was a Newton Victor GX-10 Medium Therapy X-ray Apparatus at 140 KVp (kilo-voltage peak). No filter was used; the inherent filtration of the apparatus was equivalent to 1 mm of aluminium. The source of X-ray energy was 25 cm from the chickens. The Inverse Square Law applied here, the dosage varying with the depth of the chicken from the mid-point. The dosage rate was calculated to be about 205 r. per minute.

b) Thioguanine treatment (2-amino-6-mercaptopurine)

Thioguanine (brand 50-71) was used at the rate of 60 mg per kg body weight. It was dissolved for use in weak ammonia of approximately 8 per cent and injected subcutaneously. Dilute ammonia solution was recommended

and was found better for inhibiting serological immune response than suspensions in water injected by the same route even though the former caused necrotic lesions at the focus of injection. These healed rapidly however. The drug was generously supplied free for this project by Burroughs Welcome Ltd., London.

Experiment 7

Trials with bovine serum-~~albumen~~

Preliminary dosage trials were carried out to establish the rates capable of inhibiting antibody production completely. Chickens after treatment were immunized against bovine serum according to the schedule used before, and the criterion for deciding the presence or absence of inhibition of immune response was by the haemagglutination titre, if any, obtained from immunized and control chickens. X-irradiation of the chickens was accomplished as described, but the heads of the chickens were protected. Three dosage levels were utilized: 600 r, 800 r, and 1000 r, using chickens two weeks old. Two chickens were used per group. Immunization against bovine serum was done two days after the X-ray treatment. Control chickens were not irradiated. Haemagglutination tests were carried out two weeks later on all the chickens and subsequently every fortnight for eight weeks.

Thioguanine, at 60 mg per kg body weight, was injected into two-week old test chickens two days after immunization against bovine serum. Two control chickens received no thioguanine. Serum for haemagglutination tests was taken two weeks later, and subsequently every fortnight for eight weeks.

TABLE 17 : EXPERIMENT 7

ANTIBODY LEVELS TO BOVINE SERUM FOLLOWING
X-IRRADIATION AND 6-THIOGUANINE ADMINISTRATION

Treatment	Antibody Titres			
	2nd week	4th week	6th week	8th week
600 r	80	160	80	80
600 r	160	320	160	80
800 r	40	40	0	0
"	0	20	0	0
1000 r	0	0	0	0
"	0	0	0	0
6 mg.Thiog.	0	0	0	0
"	0	0	0	20
Control	2,560	1,280	640	640
"	1,280	1,280	640	1,280

Results

The results are entered in Table 17. A treatment of 600 r did not destroy the antibody response of chickens aged two weeks. Some reduction of the titres was obtained however (1:80 - 1:160 in the second week as against 1:1280 - 1:2560 in the controls). With a treatment of 800 r, a marked reduction of response was obtained, titres in the second week being zero - 1:40. Complete inhibition was obtained in chickens given a dosage of 1000 r when no antibody was detectable. Thioguanine at the rate of 60 mg per kg body weight inhibited antibody production for eight weeks. Only one chicken recovered and showed a titre of 1:20 in the eighth week. The control chickens showed high titres (1:1280 and 1:2560 in the second week falling to 1:640 and 1:1280 in the eighth week).

Experiment 8

Experiment with Ascaridia galli

For the definitive experiment with Ascaridia galli infection as the antigenic stimulus, the X-ray dosage rate employed was 1000 r. This time whole body irradiation was carried out to offset possible recolonization by lymphoid tissue from sheltered areas to irradiated areas of the body. Irradiation was done two days before chickens in the group were infected with a daily dosage of 1000 eggs. In the second group of chickens, 6 mg of thioguanine was injected into each chicken two days after starting the infection and subsequently thioguanine treatment was carried out every three weeks till the twelfth week. This repeated injection was carried out in an attempt to prevent recovery of the antibody response since one chicken in the preliminary trial had begun to show a low antibody titre (1:20) by the eighth week. Three chickens were killed from each group each fortnight for twelve weeks and differential worm counts were done. The three remaining birds from each group were killed in the fifteenth week and their worm burden counted. Sections of the gut were cut for pathological examination. Haemmagglutination trials were also carried out with sera taken from the chickens before slaughter to measure the antibody titre.

TABLE 18

X-IRRADIATED AND 6-THIOGUANINE TREATED BIRDS RECEIVING 1,000 EGGS PER DAY: DIFFERENTIAL WORM COUNTS FOLLOWING SLAUGHTER AT FORTNIGHTLY INTERVALS

X- IRRADIATED) 6- THIOGUANINE.

	LARVAE				Adult	LARVAE				Ad.
Weeks	2nd st.	3rd st.	4th st.	Total	Total	2nd st.	3rd st.	4th st.	Total	Tot
	1,000	800	-	1,800	-	1,333	833	-	2,166	
2	1,667	333	-	2,000	-	633	67	-	700	
	500	400	-	900	-	1,933	233	-	2,166	
	3,167	1,533	-	4,700	-	3,899	1,133	-	5,032	
	167	500	-	667	-	167	433	-	600	
4	267	867	-	1,134	-	400	233	-	633	
	233	400	-	633	-	367	133	-	500	
	667	1,767	-	2,433	-	934	799	-	1,733	
	33	500	-	533	-	600	67	-	667	
6	33	433	-	466	-	67	100	-	167	
	-	767	-	767	-	133	300	-	433	
	66	1,700	-	1,766	-	800	467	-	1,267	
	33	467	-	500	-	-	433	-	433	
8	-	600	67	667	-	-	133	33	166	
	-	500	-	500	-	67	267	-	334	
	33	1,567	67	1,667	-	67	833	33	933	
	-	233	-	233	-	-	133	33	166	3
10	-	133	33	166	-	-	233	33	266	-
	-	800	-	800	-	-	300	-	300	-
	-	1,166	33	1,199	-	-	666	66	732	3
	-	600	-	600	-	-	100	67	167	1
12	-	167	33	200	4	-	167	-	167	1
	-	100	33	133	-	-	200	33	233	-
		867	66	933	4	-	465	100	567	2
	-	-	-	-	-	-	33	-	33	-
15	-	167	67	234	2	-	67	33	100	2
	-	433	-	433	1	-	100	-	100	3
		600	67	667	3	-	200	33	233	5

Results

Thioguanine and X-ray treatment completely inhibited the production of antibody. No positive haemagglutinations with helminth antigen were obtained at any time on any of the sera examined in this experiment. The results of helminthological examinations on slaughtered birds are given in Table 18. The findings show no marked divergence from the results obtained with the group challenged at the same rate as used here (1000 eggs per day) in experiment 6. Just as in that experiment, where the birds elicited a full antibody response, the present birds presented strong evidence of anthelminthic resistance. The appearance of adult worms was delayed until at least the tenth week (hence the pre-patent period must have been in excess of this although it was not confirmed by carrying out egg counts). Third-stage larvae persisted in appreciable numbers up to the twelfth week after which they began to become sparser. These findings establish quite conclusively that there is a protective reaction by the host manifesting the resistance criteria of earlier experiments in the absence of antibody production.

It was noticed that chickens treated with thioguanine showed consistently lower counts of third-stage larvae than did those of the group treated with X-rays. It is possible that theioguanine affects the metabolism of larval worms in such a way as to limit their survival.

On the other hand, adult worms recovered post-mortem in this experiment showed no appreciable size differences as between the X-irradiated and thioguanine-treated birds.

DISCUSSION

The importance of a steady supply of embryonated eggs in good condition can not be over-emphasised for a study of the nature described in this thesis. Most of the work carried out by previous authors has been based on infections set up by a single infective dose. In such work, since culture methods such as those of Riedel (1947), (1951), and Siddiqui (1954) which yield but scanty quantities of embryonated eggs are quite adequate. When, however, large doses of eggs are needed for continuous challenge, those obtained by these methods become inadequate because they provide small numbers only, and the eggs tend to lose infectivity with time due to death of larvae. Even those surviving show much depletion of their fat storage, hence are unsuitable for experiments. This view is supported by the work of Ackert *et al.* (1947), Todd *et al* (1950), and Elliot (1954) who state that loss of stored fat in infective larvae is one of the physiological factors involved in the loss of infectivity of *Ascardia galli* egg cultures. The use of the continuous-flow or chemical centrifuge for extracting freshly passed eggs from the faeces of infected chickens, as used here, proved superior to the earlier techniques. The efficiency of recovery was over 70%, and the fertile egg content exceeded 90%, whereas fertility is rarely more than 25% using the other methods. This is because *in vivo* nearly all the eggs passed by gravid worms are fertile. Large quantities of

eggs can be extracted from the faeces of infected chickens by this method.

A comparative study of embryonating media showed that although no advantage in percentage embryonation was obtained, O.1N Sulphuric acid was the best culture medium for embryonating eggs. While eggs cultured in 1% formalin showed toughening of coats, and hence probably impeded hatchability, no such effect was obtained with Sulphuric acid. This phenomenon was also described by Fairbairn (1961) who noted formalin treatment impeded the action of enzymes on the egg coats of Ascaris suis.

Several accounts have been published concerning the pathogenicity of this parasite, but most of them have been based on single infections. In the course of this work, the pathogenic effects at continuing challenge of host have been studied. The most marked effect is the failure of infected chickens to gain as much weight as the controls. The most severely affected chickens were those which received a continued high challenge of 1,000 eggs per day, and the least affected were those where the challenge was at the rate of 10 eggs per day. Chickens on a full nutritive diet containing 15% protein manifested differences of growth rate much more prominently since at lower protein levels, growth of even the control birds was severely retarded, and hence differences between them and the affected birds

were much less evident. The reduction in growth rate may be attributed to secondary sequelae as well as to the primary effects of the infection. For example, at high challenge levels, there is frequently a diarrhoea developing as early as the second week, and persisting for up to five weeks. Birds so infected suffer severe malaise and weakness, and there is a marked inappetence. This must contribute significantly to the loss of growth. During the first five weeks of continuing high challenge, the pathogenic effects may be very marked and death may result. Blood stained stool is very prominent at this period, and the damage to the epithelia of the intestine may be very marked; haemorrhagic spots are particularly well marked. The pathogenicity to chickens under low challenge is qualitatively similar but much less severe. It is likely that the pathogenic effects are due in a large degree to the presence of larvae in large numbers in the intestinal mucosa: the time when the greatest effect on growth rate is recognised coincides with that when there are heavy larval populations in the birds. This agrees with the observations of previous investigators such as Ackert (1931), Roberts (1937), Clapham (1937) and more recently Isvetaeva (1954), Ackert et. al. (1948) Kadziolka (1960). All these authors working on single infections, are agreed that recovery from the clinical syndrome

takes place after three weeks when the larvae re-enter the intestinal lumen. It was, however, not uncommon in the present work to find larvae still present in the intestinal mucosa associated with mucosal lesions even up to the sixth week in chickens fed on a diet containing 15% protein, and up to eleven weeks when the diet was of a low protein content.

The effects on blood P.C.V. and haemoglobin values were insignificant at all levels of infestation. Even the apparent haemo-concentration occurring in chickens under high repeated challenge was not significant. At the end of the fifth week, when the diarrhoea abated, there was a slight over-compensation of the blood volume due to haemo-concentration, thus giving rise to low P.C.V. values, but again neither in the P.C.V. nor the haemoglobin levels were the changes significant statistically. This supports the findings of Ackert et. al. (1946) who found no significant differences in blood haemoglobin values between control and infected chickens following a single infection of 200 eggs at 23 days old. Sadun (1950), however, described a mild anaemia and severe leucocytosis ten days after the administration of 14,000 embryonated eggs to 9 day old chickens. These chickens recovered twenty days after infection. The period of mild anaemia coincided with the period when larvae

migrated into the intestinal mucosa causing extensive damage. It must be noted, however, that the importance of Sadun's findings is gravely diminished by his statement ". . . infected chickens . . . had on the average a lower haematocrit volume both at the peak of infection and toward the time of recovery. Although fairly (sic) constant, this difference was too small to be statistically significant".

Pathogenicity of adult worms has been mentioned by several previous workers. Ackert (1920) recorded an observation regarding the number of adult worms chickens may tolerate. He noted that thirty or more worms may visibly affect, or even occasionally be fatal to grown birds. Roberts (1937) also remarked that adverse effects on growth can occur in three-month old birds harbouring 25 worms or more, while this number could be associated with emaciation and anaemia in birds as old as six months. On the other hand, he also refers to two month old birds in which a worm burden of 30 adults produced no marked effects on growth. Roberts also considered that 50 adults could produce harmful effects on laying birds. In the present project, the most harmful effects on weight gains of infected chickens were found to be connected with heavy larval populations. Adult worms did not seem to affect growth appreciably, in fact, their presence was often associated with enhanced appetite, especially of chickens on high protein. Nevertheless,

adult worms when present in large numbers (e.g. following continuing low challenge) were often a cause of obstruction of the intestinal lumen. This phenomenon was rarely seen at high challenge rates, except when the chickens were on a low protein diet without vitamin supplements. As previously mentioned, the cause of intestinal blockage is unknown. Apparently healthy birds may die after some days or even weeks of "loss of appetite" and when examined, are seen to contain vast quantities of entangled adult worms.

Infected chickens observed during this work did not show marked cellular reactions to infection. Damage to the mucosa by larvae has been mentioned already. Migration of larvae is rare and cellular infiltration occurs only when larvae fail to return to the lumen after deeper than average penetration. Sadun (1950) also failed to find much evidence of cellular response except against dead worms. Roberts (1937), however, described patches of inflammatory reaction on the surface of mucus membranes with the accumulation of histocytes and some polymorph neutrophiles. Kadziolka (1960) also stated that hyperplasia of lymph nodules and extensive eosinophlia were constant phenomena indicating cellular reaction against infection. It is rather difficult to reconcile the findings of Roberts

and Kadziolka with the results from the present study and that of Sadun. Patches of inflammatory reaction on the surface of mucus membrane were seen in this work equally in infected and non-infected young chickens. They were attributed to changes of diet and environment, for they were hardly ever seen after four weeks. This was about the age of the chickens used by Roberts for his experiment. We have referred to the fact that chickens possess numerous well developed Peyer's patches in the intestinal walls. Hyperplastic changes of these tissues were often seen in sections from both infected and non-infected chickens. The lamina propria and the sub mucosa in the vicinity of Peyer's patches were heavily infiltrated with lymphocoytes. It is thus impossible to ascribe the cause of this to infection. Eosinophilic infiltration was low: eosinophils were not seen surrounding larvae in sections. Failure to identify cellular reactions might be due to the fact that deep penetrations of larvae in Ascaridia galli infections is rare. They normally have their heads buried between the villi and often in the Brunner's glands. When larvae are trapped, especially in the lamina propria of the villi or in the glands at the base of the villi, they often degenerate and become surrounded by fibroblasts. These are the only prominent cases of cellular response noticed.

We have so far discussed the effects of the helminth population on the host which is obviously the aspect of helminthiasis, which has the most direct economic implications. For this reason, there have been many studies concerned with the pathogenicity of Ascaridia galli to poultry and on the losses in productivity sustained by the industry through parasite infection. Unfortunately, the economic pressures have tended to emphasise this aspect, so that many of the earlier researchers have paid insufficient attention to the logistics of the parasite population, during and after establishment, and to the effect on the parasite population exerted by the host. In consequence, valuable information has been lost. In turning now to consider the dynamics of the parasite populations, we have few previous works to which the present results may be compared.

In general discussions on poultry husbandry, there appears regularly the concept of "build up" of parasite populations. This carries with it the implication that the development of successive generations of parasites is a cumulative process. The most striking finding described in the present series of experiments is the difference in the development of the parasites following low and high rates of infection. When the intake of infective stages is low

there follows a substantial output of eggs. Birds which have received a daily infection of 10 eggs per day begin in from four to five weeks to pass eggs at such a rate that they contribute positively to the contamination of the environment. Hence there follows an increase in the total worm population. Presumably this process can continue for some time since infected birds can retain their adult worm populations for several months. The next generation of young birds will meet a higher rate of infection. The process of parasite increase cannot continue indefinitely, however, since we find that although they are liable to suffer more pronounced ill effects, the birds are now less likely to become agents in the further contamination of the environment. When the challenge rate increases from 10 to 100 fold the original rate, the output of worm-eggs becomes markedly depressed and delayed or is even entirely suppressed. The dynamics of infection are, therefore, manifestly more complex than is allowed in the assumptions of many of the popular ideas of poultry husbandry. The factors which modify the results from different infection rates within populations of birds of the same broad genetic constitution are:- nutritional status and probably age. Although experiments were not designed to study the effect of age (at first experience)

on the pattern of establishment of worms from different levels of repeated infection, the experiences with single infections in older birds suggest that age will play a part.

The results of Experiment 6, in which slaughter of infected birds at weekly intervals provided information on the serial changes in the parasite population, assist greatly in the interpretation of the nature of the host control mechanism. When the infection rate is as low as 10 per day, there is a development within three to four weeks of adult worms. This resembles closely the results of this and the earlier investigation of Roberts (1937) where the administration of a dose of infective eggs on one single occasion produces a patent establishment of parasites within a similar period of time. Moreover, the size of the adult populations recovered post-mortem are of a comparable order as between these single and repeated daily infections. In this respect, therefore, repeated infection by 10 eggs per day provokes no more control of the helminth population than does a single first infection. After the termination of egg administration only adult populations can be found post-mortem. While we have already recognised the comparative unreliability of the technique used for quantitative estimates in face of the

small numbers of larvae involved, the consistent non-recovery of larvae from this series of birds after six weeks is convincing evidence of their absence. This, however, taken with the declining trend in larval numbers during the course of the infection period is strongly suggestive of an increasing failure of 2nd stage larvae to become established as the experiment proceeded. In other words, there is a slight but evident growth of resistance in the host.

When the infection rate is increased to 1,000 per day, the recovery of 2nd stage larvae declines rapidly and falls to zero before the infective period of six weeks is completed. This might be considered as due to a growth of resistance by the host which is more strongly provoked by this higher infection stimulus, or it might simply be a mechanical sequel to the diarrhoea which develops with this infection rate. At all events larvae and even unhatched eggs are passed out and are recognisable in faecal smears at this period. Nevertheless, substantial numbers of larvae reach the third instar early in the course of infestation and by the end of the third week, their numerical establishment reaches its maximum and remains remarkably constant from this time until the twelfth week or later. We have already argued that this numerical equilibrium established so early in the infection is probably composed throughout of the same individuals

which became early established in the third instar. The established 3rd instar population appears, therefore, to remain unaffected by subsequent entrants, and these are now lost soon after entry, their establishment being prevented by host resistance or by the diarrhoea as suggested above or it is conceivable that their previously established fellows exert a directly antagonistic effect. The evidence lacking is here to decide between these possibilites although the enhanced establishment already described under conditions of severe malnutrition argues in favour of a resistance mechanism in the host. Whatever the reason for the non-accumulation of larvae within the host, it is hard to explain the uniform non-continuance of development for so long a period on the part of established 3rd stage larvae except as an inhibition due to resistance of the host. The occasional appearance of adults at intervals after the twelfth week may then be explained by a decline in resistance or a relaxation of the defensive mechanism of the host, as the experience of acquisition of the infection becomes more remote.

These phenomena accompanied by the paralel phenomena of increasing in antibody to helminth antigen at the time of rejection of newly acquire larvae, and which continues during the stage of equlibrium of arrested 3rd stage larvae. Adults

begin to appear when antibody titres have already shown a marked declination.

An inhibition of larval development can be inferred from the work of Roberts (1937) dealing mainly with the pathogenicity of repeated infections. He observed that ". . . in general, despite the large number of eggs fed, very few worms had survived and the majority of these were very small in size". Although Roberts did not use a challenge dose as large as that described here, he observed in birds infected with daily doses of 100 eggs that worms reach maturity in three birds only (out of five) on the forty-eighth, fifty-second and the sixty-eighth days. In groups fed 300 eggs daily only in one chicken did the worms reach maturity by the sixty-eight day. Unfortunately, Roberts carried out no digestions of the intestines for larvae counts, an inhibition of larval decelopment can only be inferred from the length of the prepatent periods reported. The spaced out maturing of adult worms reported in his work is similar to the sporadic nature of adult worms maturation found here after the infection of 1,000 eggs per day. Sadum (1948) did not repeat his infections daily and he used only three chickens, two of which harboured one worm each. Judging from the sizes of these worms, they must

have been in the 3rd larval stage, and although Sadun concluded that they came from the last dose, it is very likely that they were part of an inhibited larval population, which he failed to identify because he did not carry out serial larval counts. Roberts and Sadun, were both concerned primarily with pathogenicity and immunity with the result that comparatively little information is advanced regarding the population dynamics of the worms.

The brief study carried out on population dynamics following single infections in this work gave an interesting finding of a great variation in the rate of growth of larvae. This is indicated by the continued presence of larval populations even by the fourth week when some adults were already present. A similar finding was reported by Madsen (1962) in single infections of Ascaridia galli and Heterakis gallinarum of chickens. He refers to a bimodal distribution of lengths in the worm populations recovered. However, the emphasis given by his statistical phraseology obscures the fact that his population probably contained 3rd stage larvae (the inferior mode) and young adults (the superior mode). He observed that "it does not seem easy to give suggestions for an explanation of the bimodal distribution which corresponds completely to the phenomenon demonstrated in recent years

of 'dormant' larvae in mammalian trichostrongylids, only that it presents itself in a more extreme form". It is most likely that the bimodal nature of worm population as seen by Madsen was caused by a variation in the developmental rates of larvae resulting in populations at three to four weeks of two facies as noticed in single infections of the present work. This bimodal population arrangement has been recorded by Dunsmore (1960) for species of Ostertagia in sheep, Twohy (1956) for larvae of Nippostrongylus muris in rats and Sommerville (1956) who even identified trimodal curves in the case of Cooperia curticei in sheep.

The population consequences of life-history phenomena cannot be overlooked in studies of this nature. The life-history of this parasite has been mentioned briefly in the introduction. Various workers are all agreed on the presence of a "tissue-phase" in the life-cycle, occurring from the tenth to the eighteenth day (Ackert, 1931) or tenth to the nineteenth day (Roberts, 1937). During subsequent studies on this parasite, Ackert and his co-workers often noticed 'dimunitive' larvae flushed out with adult worms and attributed them to subsequent accidental infection. This, however, prompted them to do further studies on the "tissue-phase" of this parasite. Ackert and Tugwell (1948) reported the tissue phase to last from the third to the

twenty-fourth day, and Tugwell and Ackert (1952) found the tissue phase to last from the tenth to the twenty-sixth day. In the last study, the peak of the tissue phase was invariably found to precede that of 'lumen larvae' which suggests that the 'tissue-phase' was obligatory to the larval development. They also found growth rates of lumen larvae and 'tissue-phase' larvae to be different, for while 'tissue phase' larvae stopped growing, lumen larvae made normal growth. They showed that the lumen larvae on the twenty-second day after infection were fourth stage larvae, while those of the mucosa were the third stage. The egg dosage used by these authors lay between 100 to 500 eggs, and the highest percentage of tissue phase larvae occurred in chickens fed 500 infective eggs. Todd et. al. (1952) recorded another interesting finding about the tissue phase. They found that when two week old chickens were infected with an egg dose of 50, the tissue phase was infrequent and that most of the larvae matured without leaving the intestinal lumen. On the seventh and fourteenth days respectively, the percentage of lumen larvae to total larvae recovered were 94.3% and 89% respectively. From Tugwell and Ackert (1952) the figures for tissue phase and lumen larvae at the peak of the tissue phase was 344 and 91 respectively (79%, 21%).

Todd et. al. explained their results by postulating that larvae in the mucosa had not followed the normal course of development of the species, and suggested that larvae from newly infective eggs or from aged infective eggs perhaps under greater compulsion to undergo their partial migration into the mucosal lining of the chick than were larvae of optimum viability. Moran et. al. (1957) gave new estimates as to the degree of tissue phase to be expected from A. galli infections as 0.41% of the whole total larvae. It is quite obvious from their figures that the apparent difference between their results and those of Tugwell and Ackert as regards the number of larvae undergoing 'tissue-phase' isdue to a difference in definition of the term. Normally, A. galli larvae hardly ever penetrate deeply into the mucosal tissues but bury their heads between villi glands or the mucus layer adhering closely to the tissues. The phenomenon of 'tissue-phase' as used by Tugwell et. al. and 'mucus larvae' as used by Moran et. al. describing the same phenomenon for it is seen that more 'mucus larvae' than lumen larvae were recovered in Moran's study. Also figures by Moran et. al. on the average lengths of the 'mucus larvae' and lumen larvae show close similarity to the length of 'tissue phase' and lumen larvae of Tugwell and Ackert in that they are consistently

shorter than lumen larvae. These show that Tugwell and Ackert's 'tissue-phase' and Moran et. al. 'mucus larvae' are probably the same as are here designated 'dormant' or 'static' larvae.

Moran et. al. worked with a dose of 800 eggs and noticed 198 total worms at the fiftieth day. Sixty-three percent of these were of very small size, mainly third stage, and were recovered from the 'mucus' layer. They showed that the 'mucus larvae' could remain static without growth for up to 50 days after infection.

Comparison of these works is not very easy as they were not carried out with the same aims. Whilst Tugwell et. al. performed studies to ascertain the duration of the 'tissue phase' phenomenon, Todd et. al. carried out experiments to ascertain whether the tissue phase was a normal behavioural pattern of development of the species. Finally Moran et. al. (1957) worked to ascertain the habitat of A. galli, and a comparison of 'mucus larvae' and lumen larvae, and to ascertain how long 'mucus larvae' can occur static.

Briefly summarising these results, it is noticed that while Tugwell et. al. found the longest 'tissue phase' to be 26 days using a dosage size of 500 eggs, Moran et. al. found it to last up to 50 days using infective dosage size of

800/

800 eggs and Todd et. al. found the phenomenon to be rare and unusual, using a dosage size of 50 eggs. It is interesting to note that one important variable in those experiments was the size of the infective dose. In the present thesis at a single infection of 1000 eggs, larval population could still be identified at the fourth week when adult populations were already present. At a repeated high challenge, larvae could be seen for up to nineteen weeks. These findings in connection with the experiments reported above seem to suggest that retardation of larval development is in some way related with 'tissue phase' or 'mucus larvae' reported in the above studies, and its occurence seems to depend largely on the size of the infective dose. If this were so, the 'tissue phase' or its synonyms, all which describe variable length of sojourns of worms into the mucosa of the intestine of infected chick or closely applied to it, probably ought not be considered in a strict sense as an obligatory part of a normal life cycle of this parasite, but rather as a reaction to factors of resistance inherent in chickens, and controlled to a large extent by a complex dynamic interplay of infective dosage size and the chickens. A similar view has been voiced by Madsen (1962) after noting bimodal/

bimodal growth curves in A. galli and Heterakis gallinarum infections of chickens, and also in the light of similar findings in mammalian trichostrongylids. One factor however which weakens this postulate is the lack of a strong cellular response against larvae encountered in histological examinations during the study. This might be because penetration was not deep enough. It was noticed that trapped larvae have fibroblasts surrounding them.

Although this is the first description of the inhibition of larval development as an important phenomenon in connection with the population dynamics of Ascaridia galli infections it is by no means a new phenomenon in nematode infections of various hosts especially of sheep. Among these are Oesophogostomum dentatum and Hyostrongylus rubidus in pig (Kotlan, 1949), Trichostrongylus retortaeformis in the rabbit (Michel, 1952), Cooperia curticei (Sommerville, 1960), D. viviparus in cattle (Taylor and Michel, 1952), Haemonchus contortus in sheep (Field et. al. 1960), Ostertagia ostertagi in cattle (Michel, 1963).

Inhibition of larval development should be distinguished from/

from the stunting effect observed by several workers in immune animals. A true developmental inhibition arrests the metamorphosis of the larvae at a precise metamorphic stage, and this appears to vary with the species of nematode. Whilst in the present work with A. galli arrest of larval development is seen to occur at the third stage of larval development, in Ostertagia ostertagi at the fourth larval stage (Michel 1963) whilst in Dictyocaulus viviparus it occurs on the fifth larval stage (Michel, 1955). When compared with other nematodes it appears that different factors operate in initiating inhibition, for whilst in the present work, assuming adequate levels of nutrition, inhibition depends mainly on dosage size irrespective of whether it is given as a single or continuing challenge. The only difference between single and continuing challenge in this connection is the extent and the duration of the inhibition resulting. In O. ostertagi Michel (1963) the host acquires the ability to inhibit development only as a result of prolonged exposure to infection, supplemented by the presence of adults.

The inhibition recorded here is not permanent. Resumption of development occurs sporadically later, but what causes it is not known. It is likely, however, that the/

the internal environment of the host plays the decisive role as not all the chickens develop adults at the same period and also not all the larvae inhibited in a chick develop at once. The epidemiological significance of this is the carrying over of the infection from one season to another, thus extending infection over a wider period of time than would have been the case if inhibition had not occurred. It is impossible to say how far the phenomenon of self-cure occurs in Ascaridiasis of Poultry. This term was first introduced by Stoll (1929) to describe the spontaneous elimination of infection of Haemonchus contortus in sheep. Stewart (1950) used the term to denote the elimination of an existing infestation in response to a heavy dose of larvae. He postulated that in natural infections the occurence of self-cure in summer in Australia after the rains when development of H. contortus was favoured indicated that the intake of infective larvae was the cause. Elimination of adult worms during the course of this study was noticed. In no case however was this a result of superinfection with infective eggs. However, only in one experimental group (low challenge) were adult worms already developed when challenge was still continuing. The dosage rate employed (10) eggs) was then clearly far short of massive./

massive. On the other hand, it was noticed that birds are very sensitive to sudden changes of environment. Movement to new batteries or cages, and slight starvation often led to elimination of worms. This however can hardly be regarded as self-cure.

Although slight acquired resistance is indicated by the numbers of adults recovered from chickens receiving 1000 eggs as a challenge dose after previous experience of infection by continuing challenge. When judged by the length of the prepatent period, however, there appeared to be no resistance developed: the prepatent periods were the same as those in chickens which had not sustained a previous infection. Nevertheless as we already mentioned this could have been due to the effect of anthelminthic treatment before infection.

Several workers, using information based on experiments with single infection as first challenge, reported strong acquired resistance to A. galli infections. Their findings were based on counts done only 3 weeks after an infection and such results as obtained cannot be said to reflect accurately the situation which would have obtained had the worms been allowed to develop to patency. Moreover, workers such as Graham et. al. (1932) and Sadun (1948) arrived/

arrived at their conclusions by using the lengths of the worms obtained after a challenge dose to decide the infection from which the worms had come. The recognition of inhibited development of larvae makes the appraisal of information derived from such works difficult.

Experience of previous infection never brought about a complete resistance to reinfection in the present work. Age resistance however seems to be operative. Whilst the prepatent period of chickens aged 2 weeks at infection was 36 days, chickens 14 weeks at infection showed a prepatent period of 51 days. Chickens of 22 weeks old at infection never developed a patent infection. That there was establishment was shown by a chick from this group which died accidentally on the ninth week while harbouring 167 larvae of third stage. This agrees with the findings of Kerr (1955) who showed that age of chickens affect the prepatent period of the infection. Epidemiologically, arguments for acquired resistance in this parasite is academic. Although a measure of resistance is shown to occur as a result of a previous infection, there are however enough worms developing as to constitute a strong source of infection to a susceptible flock. This suggests the findings of Kozar (1948) who stated that previous infestation of Ascaridia columbae in pigeons does not bring about a complete resistance. An

important/

important sequel of the extended prepatent period as seen under age resistance is the prolongation of patency in an infection resulting in less eggs being available for infecting chickens.

In the field, chickens which pick up repeated high doses of infective eggs are capable of carrying over dormant larvae to an advanced age before patency occurs. In chickens picking up low infections, adults develop early, and most of the worms may be lost by the seventh month, or else the fecundity will be greatly reduced. In the first case and possibly in the second, old birds would still harbour worms. Age immunity thus operates mainly against infections of first experience and has little or no effect on already established adult worms. This might explain the findings of Wakelin (1964) and a personal observation during a survey in Nigeria where often healthy adult chickens in the field were seen to harbour larger populations of adult worms than would be expected if age immunity were operative.

An antibody basis of immunity to A. galli infections was postulated as early as 1932 by Graham et. al. It has not hitherto been backed up by a demonstration of the antibody by conventional methods although Sadun (1947, 1949) was able to demonstrate precipitates around natural openings of/

of larvae immersed in sera from infected chickens. The significance of antibody production in helminth infections has been a subject of speculation for a long time. The formation of precipitates at the orifices of the larvae of some parasites when incubated in sera from infected hosts has led to the belief that an antigen-antibody reaction is concerned in resistance to helminth infestations. This view was based on the belief that the defensive mechanism of the body reacts to helminth infestations in the same way as in bacterial and viral infections. This theory of combating helminth infection was set forth by Taliaferro and Sarles (1939) working on Nippostrongylus in rats. They theorized that such precipitates might interfere in some manner with the successful persistence of the parasites in the host due to a mechanical blockage of the important body openings. Since the formation of this theory several workers such as Campbell (1955), Chute (1956) Silverman and Patterson (1960), Soulsby (1960) etc. have drawn similar conclusions as to the importance of the antibody in the resistance phenomenon.

In the field of A. galli infections, Sadun (1949) stands out as a major exponent of this thesis. He injected chickens intraperitoneally with immune serum at the rate of

6/

6 ml. per 100 gms. of body weight (equivalent to almost 2/3 of the actual blood volume of the chicken) and infected them with 3,000 eggs each, at the age of 32 days. These chickens were killed 22 days later. Two groups of control chickens were used, one group receiving equal amount of normal serum and the other group none. The criteria he used for determining the presence of immunity was number and size of worms recovered post mortem and the ability of chickens to survive lethal doses which he had worked out in 1949. He found chickens injected with immune serum to harbour less worms than the two control groups. In a second experiment, chickens with passive immunity were able to survive lethal doses of infection. From these results, he concluded that resistance of chickens to A. galli was due to antibody production. Egerton and Hausen (1955) also tried passive transfer of immune serum to chickens and on infection found these chickens to harbour more worms than the controls at the end of three weeks. Also these chickens gained significantly more weight than the controls at 10% significant levels. This work was interesting because these workers introduced a new concept of immunity in parasitic infections. The immune serum was obtained from chickens fed 100 eggs and bled three weeks later. They used the term immunity to denote resistance to the effects of parasitism and claimed that the chickens receiving immune serum were protected/

protected because they gained more weight than the controls even though they harboured more worms. They postulated that the antibodies they injected into these chickens were able to neutralize toxic products of the worms, and effectively prevent the detrimental effects of these on the host, thus allowing them to make normal growth. They finally concluded that the manifestation of immunity to A. galli in chickens is the ability to allow an increased number of worms to become established without adverse effects being suffered. This seems to represent a stretching of the expression immunity to embrace the precise opposite to what is normally meant namely a suppression or containment of the infective agent. An assumption made by them is that chickens infected with 100 A. galli eggs have antibodies in their blood stream three weeks later. From our findings only minimal antibody titres (1/20) are present at the third week after a single infection of 1000 eggs. Moreover the results of Egerton and Hausen are the reverse of those obtained in the present work where chickens under a continuing challenge of 1000 eggs harboured plenty of inhibited larvae and gave high antibody titres but failed to gains as much weight as the controls or birds with lower infective dosages.

Considering the work of Sadun (1949), if antibody is able/

able to protect chickens by passive transfer as assessed by reduction of worm numbers and size, the logical corollary is that more worms will establish in the host and develop without inhibition in the absence of antibody. Experiments reported in the thesis have demonstrated conclusively that X-ray and thioguanine treatment are able to inhibit completely the antibody production of treated chickens. Infected chickens treated with these substances failed to produce antibody against the infection of A. galli yet there occurred neither reduction of inhibited larval populations nor an increase in the rate of metamorphosis of larval population to adult populations as would be expected if the antibody were agentive in resistance against the infection. Although there was a reduction in the larval population of chickens treated with thioguanine, there was no increase in the adult population so there was no increased metamorphosis of third stage larvae to the adults. The decrease of the third stage larvae could then be due to the effect of thioguanine treatment since it is known to be an antimetabolite interfering with stages in nucleic acid synthesis. This might have had some effect in the metabolism of the developing worms thus killing some of them. On the other hand X-irradiation was without obvious effect on the subsequently entered/

entered helminths.

These results show conclusively that antibody production, at least as measured in the present experiments plays no effective role in the resistence of chickens to infection with A. galli. Neither X-ray nor thioguanine treatment affected goblet cells production in treated chickens. In view of the present findings, the only importance attributable to the titres obtained in the present work is that they reflect in a way a measure of the activity of the parasites in the host since chickens under a continuing challenge of 1000 eggs showed the highest titres. The very low titres noticed in low protein experiments, however, show that chickens are best able to reflect the activity of the parasites under good nutritional conditions. The significance of high goblet cell counts can only be said to reflect the degree of irritation caused by the worm populations.

Distinction should be made between resistance of the host to parasitic infection and resistance of the host to the effects of the parasitic infections. If we accept the finding of Herrick (1926) that transfer of serum to infected chickens makes them in general more resistant to the effects than to the worms themselves, then the results of Egerton and Hausen might be so interpreted. On the other hand the findings of Sadun (1949) are impossible to reconcile with those recorded here.

ACKNOWLEDGEMENTS

I am greatly indebted to the Department of Technical Co-operation under whose fellowship this project was carried out, and also to the Federal Government of Nigeria for leave of absence to carry out the work.

I am extremely grateful to Dr. J. Allan Campbell who supervised and guided me throughout the project and to the entire staff of the Zoology Department especially the Sub-department of Parasitology, for their ready advise when approached. I wish also to thank Dr. Bolton of P.R.C. for advise on nutrition and Dr. Selman of the Department of Animal Genetics, Edinburgh University for invaluable help in irradiation of chickens.

Finally I wish to convey my gratitude to the Ministry of Agriculture, Fisheries and Food, Lasswade Poultry Laboratories, for the supply of chickens used here.

APPENDIX

1. Wintrobes anti-coagulant.

Potassium oxalate	2 gr.
Ammonium oxalate	3 gr.
Distilled water	100 ml.

2. Formol-Saline-Sublimate.

Solution A (Formol-Saline)

Formalin (40% formaldehyde)	50 ml.
Sodium chloride	4.5 g.
Distilled water	450 ml.

Solution B (Formol sublimate)

Saturated Aqueous mecuric chloride	450 ml.
Formaldehyde (40% Formalin)	25 "
Glacial Acetic Acid	5 "

Mix Solutions A and B in the ratio of one to one.

3. Bouin's solution.

Saturated Aqueous Picric Acid	75 ml.
Formalin (40% Formaldehyde)	25 "
Glacial Acetic Acid	5 "

4. Delafields Haematoxylin

Haematoxylin crystals	4 gm.
Absolute Alcohol	25 ml.
Saturated Ammonium alum	400 ml.
Glycerine	100 "
Methyl alcohol	100 "

5/

5. Eosin

Eosin	0.5 g.
96% Alcohol	100 ml.

6. Meyer's Mucicarmine

Aluminium chloride	0.5 g.
Carmine	1.0 g.
Distilled water	2 ml.

Boil for 2 mins. and make up to 100 ml. with 50% alcohol.

7. Alsever's solution

Dextrose	2.05 g.
Sodium citrate	0.8 g.
Sodium chloride	0.42 g.
Distilled water	100 ml.

Adjust pH to 6.1 using 10% Citric acid solution.

8. Biuret Reagent

Sodium Potassium Tartrate	45 g.
Copper Sulphate ($CuSO_4.SH_2O$)	15 g.
Potassium iodide	5 g.
Sodium hydroxide 0.2N.	1000 ml.

BIBLIOGRAPHY

Ackert, J.E. (1920)
Studies on the development of Ascaridia perspicillum parasitic in fowls. Anat. Rec., XVII : 331

_____ (1923)
On the life-history of the Fowl nematode Ascaridia perspicillum. Anat. Rec., XXVI : 359.

_____ (1930)
Recent developments in the importance and control of the intestinal round worm. Ascaridia lineata of Chickens. Fourth World's Poultry Congress, London, 454.

_____ (1931)
The morphology and life-history of the fowl nematode Ascaridia lineata. Parasitiology, 23 : 360

_____, Cooper, R.M. and Dewhirst, L.M. (1947)
Viability of Ascaridia eggs under varying conditions of age and administration. Trans. Amer. Micros. Soc. 66 : 383

_____, and Edgar, S.A. (1938)
Goblet cells and age resistance to parasitism.
J. Parasitol. 19 : Suppl. 13.

_____, Eisenbrandt, L., Wilmoth, J.H. Glading, J. and Pratt, I. (1935)
Comparative resistance of five breeds of chickens to Ascaridia lineata. J. Agric. Res. 50 : 607

_____, Edgar, S.A. and Frick, L.P. (1939)
Goblet cells and age resistence of animals to parasitism.
Trans. Am. Micros. Soc., 58 : 81

_____, Fisher, M. and Zimmerman, N. (1927)
Resistance to parasitism affected by fat soluable Vitamin A.
J. Parasitol. 13 : 219

_____, Graham, G.L., Nolf, L.O. and Porter, D.A. (1931)
Quantitative studies on the administration of variable numbers of nematode eggs (Ascaridia lineata) to chickens.
Trans. Amer. Micros. Soc. 50 : 206.

_____, and Gaafar, S.M. (1949)
Phosphorus defficiency a limiting factor in fowl parasitism.
J. Parasitol, 35 : Suppl. p. 11.

_____ and Herrick, C.A. (1928)
Effecting/

Effects on the nematode Ascaridia lineata on growing chickens. J. Parasitol. 15 : 1.

_____, Porter, D.A. and Beach, T.D. (1935)
Age resistance of chickens to the nematode Ascaridia lineata. J. Parasitol. XX I : 205.

_____ and Tugwell, R. (1948)
Tissue phase of Ascaridia galli life cycle elucidated by artificial digestion apparatus. J. Parasitol., 34 : Suppl. p. 32.

_____ and Wisseman, C.L. (1946)
Tolerance of fowls for nematode infections of intestinal helminths. Am. J. Trop. Med. 26 : 721.

_____ and Whitlock, J.H. (1935)
Studies on Ascarid nutrition. J. Parasitol. 21 : 428.

_____, Whitlock, J.H. and Freeman, A.E. (1940)
The food of the fowl nematode Ascaridia lineata (Schneider). J. Parasitol. 26 : 17.

Alexander, B. (1961)
Fundamentals of Radiobiology. Pergamon Press.

Allison, J.B. (1953)
Dietary Proteins - their functions in health and disease. J. Agric. Food Chem. 1 : 71

Allison, J.B. (1955)
Biological evaluation of Proteins.
Physiol. Revs., 35 : 664.

Baylis, H.A. (1932)
What is the common large round worm of chickens.
Ann. Mag. Nat. Hist. (Ser. 10), X : 520.

Berenbaum, M.C. (1962)
The effects of cytotoxic agents on the production of antibody to T.A.B. vaccine in the mouse. Biochem. Pharmacol. 11 : 29.

_____ and Brown, I.N. (1964)
Dose-response relationships for agents inhibiting the immune response. Immunol. 7 : 65.

Borduas/

Borduas, A. and Grabar, P. (1953)
L'hemagglutination passive dans la recherche des anticorps antiproteiques. Ann. Inst. Pasteur, 84 : 903.

Boyden, S.V. (1951)
The absorption of proteins on erythrocytes treated with tannic acid and subsequent haemagglutinations of antiprotein sera. J. exp. Med., 93 : 107.

Bradshaw, G. (1907)
Diseases of fowls - Internal parasites.
Agric. Graz. N.S. Wales, 18 : 799.

Brading, I. (1956)
The use of tannic acid to link blood group substances to Group O red cells. Aust. J. exp. Biol. med. Sci. 34 : 157

Cancer Chemotherapy Reports (1961)
Thioguanine and Thioguanosine. Cancer Chemotherapy National Service Centre. U.S. Dept. of Health Education and Welfare. Pub. Health Service 202 - 212.

Campbell, C.H. (1955)
The antigenic role of the excretions and secretions of Trichinella spiralis in the production of immunity in mice. J. Parasitol., 41 : 483.

Campbell, J.A. and Gardiner (1961)
Personal communication.

Chandler, A.C. (1953)
Immunity in Parasitic diseases. J. Egyptian Med. Ass., 36 : 811.

Chute, R.M. (1956)
The dual antibody response to experimental trichinosis.
Proc. Helminth. Soc. Wash., 23 : 49.

Clapham, P.A. (1937)
On some lessions associated with helminths in birds of economic importance. J. Helminth. 15 : 49.

Cleg, J.W. and King, E.J. (1942)
Estimation of haemoglobin by the Alkaline haematin method.
British Med. J., 2 : 329.

Cobb, N.A. (1896)
Wormy fowls. Agric. Graz. N.S. Wales. 7 : 746.

Cok/

Cok, L. and Farrell, V.R. (1955)
A method for coupling protein antigens to erythrocytes. Description of methods. J. exp. Med. 102 : 631.

Cram, E.B. (1927)
Bird parasites of the Nematode Sub-orders, Strongylata, Ascaridata and Spirurata. U.S. Nat. Mus. Bull. 140 : 82 and 93.

Csizmas, L. (1960)
Preparation of formolinized erythrocytes.
Proc. Soc. exp. Biol. (N.Y.) 103 : 157.

Dixon, F.J., Talmage, D.W. and Maurer, P.H. (1952)
Radiosensitive and radio-resistant phases in the immune response. J. Immunol. 68:693.

Dujardin, F. (1845)
Histoire naturelle des Helminthes ou vers intestinaux.
654 pp. Paris.

Dunsmore, J.D. (1960)
Retarded development of Ostertagia species in sheep.
Nature, (London) 186 : 986

Eisen, B., Demis, D. and Crosby, W.H. (1962)
Thioguanine therapy, systematic lupus erythematosus, atopic dermatitis and other malignant diseases.
J. Amer. Med. Assoc. 179 : 789.

Eisenbrandt, L.L. and Ackert, J.E. (1940)
On the resistence of chickens to the intestinal nematode Ascaridia lineata (Schneider) following immunization.
Am. J. Hyg. 32 : 1.

Elliot, A. (1954)
Relationship of ageing, food reserves and infectivity of larvae of Ascaridia galli. Exptl. Parasitol.
3, (4) : 307.

Fairbairn, D.D. (1961)
The invitro hatching of Ascaris lumbricoides eggs.
Canadian J. Zoology. 39 : 153.

Fiset, L. (1962)
Non precipitating rabbit antibodies: a study of their reactivity invitro by means of the quantitative precipition and tannic acid haemagglutination techniques.
Immunol. 5 : 580.

Flick/

Flick, J.A. (1948)
Use of formalin-treated red cells for the study of influenza A virus haemagglutination activity. Proc. Soc. Exp. Biol. (N.Y.) 68 : 448.

Freeborn, S.B. (1923)
Nicotine as a poultry vermifuge. Science, 57 : 692.

Freund, J. (1931)
On the mechanism of toxin-antitoxin reactions. J. Immunol. 21 : 127.

Frenzen, K. (1955)
Beitrag zur morphologie und synonymie von Ascaridia galli. Zeitschrift fur Parasitenkunde, 17 : 93.

Frick, L.P. and Ackert, J.E. (1947)
Nature of duodenal nematode growth-inhibiting factor. J. Parasitol. 33 : Suppl. p. 19.

_____ (1948)
Further studies on duodenal mucus as a factor in age resistance of chickens to parasitism. J. Parasitol. 34 : 192.

Fröhlich, I.A. (1789)
Beschreibungen einiger neuen Eingeweidewurmer. Naturforscher, Halle, Bd. 24 : 101.

Gaafar, S.M. and Ackert, J.E. (1953)
Studies on mineral deficient diets as factors in resistance of fowls to parasitism. Exptl. Parasitol. 2 : 185.

Germuth, F.G. and Ottinger, B. (1950)
Effect of 17-Hydroxy-11-Dehydrocoricosterone (Compound E) and ACTH on Arthurs reaction and antibody formation in the rabbit. Proc. Soc. exp. Biol. (N.Y.), 74 : 815.

Goodman, M., Wolfe, H. and Norton, S. (1951)
Precipition production in chiekens, VI. The effect of varying concentrations of sodium chloride on preciption formation. J. Immunol., 66 : 225.

Graham, G.L. Ackert, J.E. and Jones, R.W. (1932)
Studies on an acquired resistance of chickens to the nematode Ascaridia galli. Am. J. HYg. XV (3) : 762.

Hansen, M.F., Olson, L.J., and Ackert, J.E. (1954)
Improved techniques for culturing and administering ascarid eggs/

eggs to experimental chickens. Expt. Parasitol., 3 : 464.

Hektoen, L. (1918)
The production of precipitins by the fowl. J. Infect. Dis., 22 : 561.

Herrick, C.A. (1926)
Studies on the resistence of chickens to the nematode Ascaridia perspicillum. Am. J. Hyg. 6 : 153.

Ingraham, J.S. (1958)
The preparation and use of formolinized erythrocytes with attached antigens or haptens to titrate antibodies. Proc. Soc. exp. Biol. (N.Y.) 99 : 452.

Jacobs, L. and Lunde, M. (1957)
Haemagglutination tests for toxoplasmosis. Science, 125 : 1035.

Kagan, I.G. and Bargai, U. (1956)
Studies on serology of trichinosis with haemagglutination, agar diffusion and precipitin ring tests. J. Parasitol. 42 : 237.

Kadziolka, A. (1960)
(Histopathological studies on the tissue test in experimental Ascaridiosis in chickens). Acta Parasitologica Polonica, 8 : 315.

Kerr, K.B. (1955)
Age of chickens and the rate of maturation of Ascaridia galli. J. Parasitol. 41 : 233.

Kotlan, A. (1949)
The developmental and pathological significance of the histotrophic phase in parasitic nematodes. Rep. 14th Internat. Vet. Congr. 2 : 61.

Koutz, F.R. (1953)
The effect of built up litter on the parasitic ova and oocysts of poultry. Poultry Science, 32 : 313.

Kozar, Z. (1948)
Age acquired resistance and influence of diet and light on the parasite Ascaridia columbae in pigeons. Bulletin of the Institute of Marine and Tropical Med of the Medical Academy in Gdansk, Poland. 1 : 39.

Linstow/

Linstow, O. (1906)
Nematoden des zoologischen Museum in Konigsberg.
Arch. Naturgesh., 72 : 249.

Madsen, H. (1962a)
On the interaction between Heterakis gallinarum, Ascaridia galli, 'Blackhead' and the chicken.
J. Helminth., 36 : 107.

_____ (1962b)
The so-called tissue-phase in Nematodes.
J. Helminth. 36 : 143.

Magalhaes, P. (1892)
Notes d'Helminthologie brasilienne. 2. Heterakis brasiliensis de la poule domestique. Bull. Soc. Zool. France, 17 : 219.

Makinoden, T., Grengozian, N. and Canning, R. (1960)
Demonstration of a normal serum macroglobulin co-precipitating with bovine serum albumin (BSA) - chicken anti B.S.A.
J. Immunol., 85 : 439.

McKenna, J.M. (1957)
A stable preparation of antigen-sensitized erythrocytes.
Proc. Soc. exp. Biol. (N.Y.) 95 : 591.

Michel, J.F. (1952)
Inhibition of development of Trichostiongylus retortaeformis.
Nature (Lond.) 169 : 933.

_____ (1955)
Studies on host resistance to Dictyocaulus infection. 1. The phenomenon of inhibited development.
J. Comp. Path., 65 : 149.

_____ (1963)
Host resistance and infection of Ostertagia ostertagi in calves. Parasitology, 53 : 63.

Moran, J.F. and Mizelle, J.D. (1956)
Notes on the habitat and tissue-phase of Ascaridia galli (Schrank 1788). J. Parasitol., 42 (4 Sect. 2) : 18.

_____ and _____ (1957)
Studies on Ascaridia galli (Schrank 1788). American Midland Naturalist, 58 : 170.

Nutritional requirements of domestic animals . 1 Nutrient requirements of Poultry. National Academy of Sciences, National Research Council Publication 827 (1960).

Orlans/

Orlans, E., Rose, M.E., and Marrack, J. (1961)
Fowl antibody: 1. some physical and immunochemical properties. Immunology, 5: 262.

Oliver-Gonzalez, J. (1946)
Functional antigen in helminths.
J. Infect. Dis., 72 : 202.

Owen, R.W. (1951)
The helminth parasites of domesticated birds in mid Wales.
J. Helminth., 25 : 105.

Pande, P.G. and Krishnamurty, D. (1959)
Interelationship between hypovitaminosis A and Ascaridia galli infestation in Poultry. Poultry Science, 38 : 13.

Philips, F.S., Sternber, S.S., Hamilton, L.D. and Clarke, D.A. (1956)
Effects of Thioguanine in mammals. Cancer, 9 : 1092.

Railliet, A. et Henry, A. (1912)
Quelques nematodes parasites des reptiles.
Bull. Soc. Path. Exot. 5 : 521.

_____ et _____ (1914)
Essai de classification des Heterakidae.
Congr. Internat. Zool. Monaco, 1913, 674.

Reid, W. (1944)
The effect of host starvation on worm elimination and glycogen depletion with the nematode Ascaridia galli.
J. Parasitol., 30 : Supple. p. 12.

_____ (1960)
Effects of temperature on the development of the eggs of Ascaridia galli. J. Parasitol., 46 : 63.

Reinder, L. and Fischer, O. (1929)
Beitrage zum Mechanismus der Immunkorper wirkung. 1. Mitteilung. Die rolle des Ambozeptors und des Komplements bei Zytolyse. A. Immun. - Forsch. 61 : 317.

Riedel, B.B. (1947)
New technique of culturing and feeding Ascarid eggs.
Trans. Amer. Micro. Soc. 66 (4) : 396.

_____ (1951)
A simplified method of culturing Ascarid eggs. Trans. Amer. Micro. Soc. 70: 57.

Roberts/

Roberts, F.H.S. (1937)
Studies on the biology and control of the large round worm of fowls Ascaridia galli (Schrank 1788) Freeborn 1923. Queensland Dept. Agric. and Stock. Bull. 2.

Ross, J.G. and Armour, J. (1960)
The significance of faecal Egg Counts and the use of Serum Albumin levels and packed cell volume percentages to assess Pathogenicity in helminthiasis. Vet. Rec. 72. 137.

Rudolphi, C.A. (1803)
Neue Beobactungen uber die Eingeweidewurmer. Arch. Zool. und Zool. 2 : 1.

Sadun, E.H. (1947)
Immunity in chickens to Ascaridia galli. J. Parasitol. 33 (6 Sect. 2) Suppl. pp. 13-14.

_____ (1948)
Resistance induced in chickens by infections with the nematode Ascaridia galli. Am. J. Hyg. 47 : 282.

_____ (1949)
The antibody basis of immunity in chickens to the nematode Ascaridia galli. Am. J. HYg. 49 (1) : 100.

_____ (1950)
The influence of dietary pteroyglutamic acid and of A.P.A. liver extract on the survival and growths of the nematode Ascaridia galli in chickens fed purified and natural diets. Am. J. Hyg. 51 : 274.

_____ (1950)
Studies in the pathogenicity in chickens of single infections of variable size with the nematode A. galli. Poultry Science 29 : 712.

Sartorelli, A.C. and Le Page, G.A. (1958)
Metabolic effects of 6- Thioguanine 11. Biosynthesis of nucleic acid Purines in vivo and in vitro. Cancer Res. 18 : 1329.

Schrank, F.P. (1788)
Verzeichniss der bisher hinlanglich bekannten Eingeweidewurmer, nebst einer Abhandlung uber ihre Anverwandschaften. Munchen, 116 pp.

Scot, J.W. (1913)
A new means of transmitting the fowl nematode Heterakis perspicillum. Science XXXVIII (984) : 672.

Schneider/

Schneider, A. (1866)
Monographie der Nematoden. 357 pp. Berlin.

Schwartz, R., Stack, J. and Damashek, W. (1958)
Effect of 6-mercaptopurine on antibody production.
Proc. Soc. exp. Biol. (N.Y.) 99 : 164.

Schwartz, B. (1925)
Ascaridia lineata, a parasite of chickens in the United States. J. Agric. Res. 30 : 763.

Shikhobalova, N.P. and Kustova, L.I. (1950)
(Influence of Ascaridae on the amount of the reserve of vitamin A in the liver of Chickens). Trudi Gelmintologcheskoi Laboratorii. Akademii Nauk S.S.S.R. 4 : 5.

Siddiqui, E.H. (1954)
A simple method of culturing ova in the body of the nematode Ascaridia galli. Proc. Pakistani Science. Congr. (Abstract) 6th. (1954) p. 229.

Silverman, P.H. and Patterson, J.E. (1960)
Histotrophic (Parasitic) stages of Haemonchus contortus.
Nature, Lond. 185 : 54.

Sommerville, R.E. (1960)
The growth of Cooperia curtecei, a nematode parasite of sheep.
Parasitology, 50 : 261.

Soulsby, E.J. (1956)
Studies on the serological response in sheep to naturally acquired gastro-intestinal Nematodes. 1. Prepration of Antigen and evaluation of serological technique.
J. Helminth, 30 : 129.

_____ (1957)
Studies on the serological response in sheep to naturally acquired gastro-intestinal nematodes. 11. Responses in a low ground flock. J. Helminth, 31 : 145.

_____ (1960)
Use of Haemagglutination technique for the detection of antibody in gastro-intestinal nematode infections of sheep.
British Veterinary Journal 116 : 309.

_____ and Sommerville, R. and Stewart, D. (1959)
Antigenic stimulus of exsheathing fluid in self-cure of sheep infected with Haemochus contortus. Nautre, Lond.
183 : 553.

Spur/

Spur, C.L. (1947)
Influence of Nitrogen mustards on the antibody response. Proc. Soc. exp. Biol. (N.Y.) 64 : 259.

Stafseth, H. and Thompson, W. (1932)
The effects of treatment, and prevention of worm infection in Poultry. J. Am. Vet. Med. Assoc. LXXX : 467.

Sterzl, J. (1961)
Effect of some metabolic inhibitors on antibody formation. Nature (Lond.) 189 : 1022.

Stewart, D.F. (1950)
Studies on resistance of sheep to infection with Haemonchus contortus and Trichostrongylus spp. and on the immunological reactions of sheep exposed to infection. IV. The antibody response to natural infestation in grazing sheep and self-cure phenomenon. Austr. J. Agr. Res. 1 : 427.

Taliaferro, W.H., and Sarles, M.P. (1939)
The cellular reactions in Nippostrongylus murris. J. Infect. Dis. 64 : 157.

_____ , Taliaferro, L.G. and Janssen, E.F. (1952)
Localisation of X-ray injury to the initial phases of antibody response. J. Infect. Dis. 91 : 105.

Taylor, E.L. and Michel, J.F. (1952)
Inhibited development of Dictyocaulus larvae in the lungs of cattle and sheep. Nature (Lond.) 169 : 753.

Thorson, R.E. (1951)
The relations of the secretions and excretions of the larvae of Nippostrongylus muris. to the production of protective antibodies. J. Parasitol., 37 : suppl. p.18.

Todd, A.C. and Crowdus, D.H. (1952)
On the life history of Ascaridia galli. Trans. Amer. Micros. Soc., 71 : 282.

Travassos, L. (1919)
Contribuicao para a Sistematica des Ascaroidea. Arch. da Esc. Sup. de Agric. e Med. Veter. Nietheroy, 4 : 15.

Tsvetaeva, N.P. (1954)
(Pathology of the early stages of Ascaridiases of chickens.) Trudi Gelmintologicheskoi Laboratorrii. Akademiya Nauk S.S.S.R., 7 : 304.

Tugwell/

Tugwell, R. and Ackert J.E. (1950)
Further studies on the tissue phase of the life cycle of Ascaridia galli. J. Parasitol. 36 : suppl. p. 16.

_____ and _____ (1952)
On the tissuse phase of the life cycle of the fowl nematode Ascaridia galli (Schrank) J. Parasitol. 38 : 277.

Twohy, D.W. (1956)
The early migration and growth of Nippostrongylus muris in the rat. Am. J. Hyg. 63 : 165.

Wakelin, D. (1964)
A survey of the intestinal helminths parasitic in British domestic fowl. J. Helminth. XXXVIII : 191.

Wells, P.D. (1962)
Mast cells, eosinophil and histamin levels in Nippostrongylus brasiliensis infected rats. Expt. Parasitol. 12 : 82.

Yorke, W. and Maplestone, P. (1962)
The Nematode parasites of vertebrates. London.

Zeder, J.G. (1803)
Anmerkungen herausgegeben. XX + 320 Leipzig.

Field, A.C., Brambell, M. and Campbell, J.A. (1960)
Spring rise in faecal worm-egg counts of housed sheep, and its importance in nutritional experiments.
Parasitology, 50: 387.

Weichselbaum, T.E. (1946)
Biuret reactions for total protein: An accurate and rapid method for the determination of protein in small amounts of blood serum and plasma.
Am. J. Clin. Pathol. (Tech. Sect.) 10:40.

Plate 1a: Formalinized sheep Red cells (1/6 objective).

Plate 1b: Formalinized Poultry Red cells 1/6 objective).

a

b.

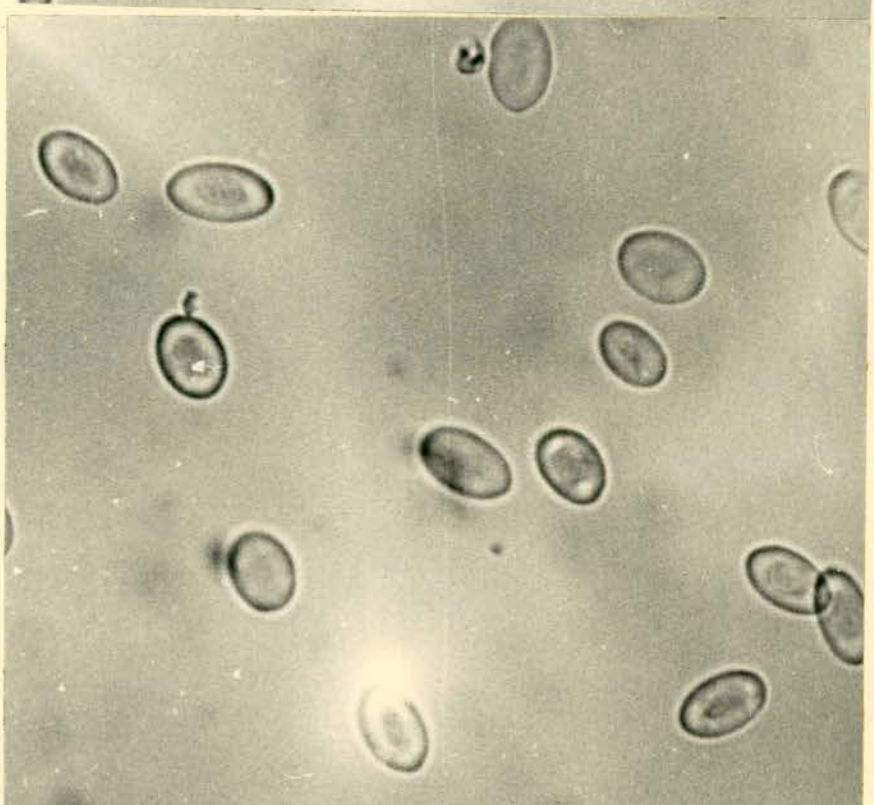


Plate 11: Intestinal obstruction (Experiment 3).
Plate 12: Section of obstructed intestine shOwing atrophy of mucous membrane.

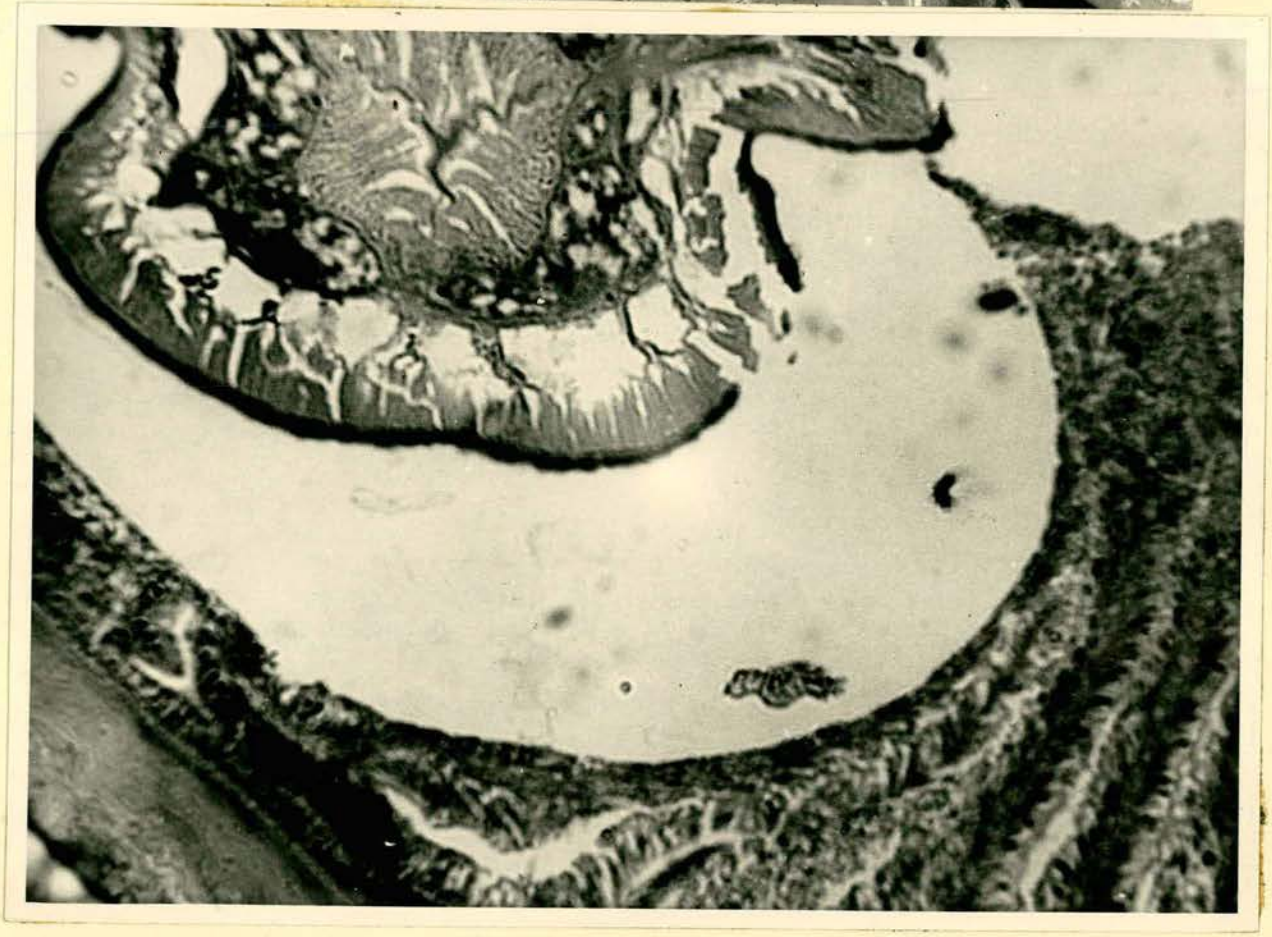


Plate IO: Adult wormsin caecum. (Experiment 3).

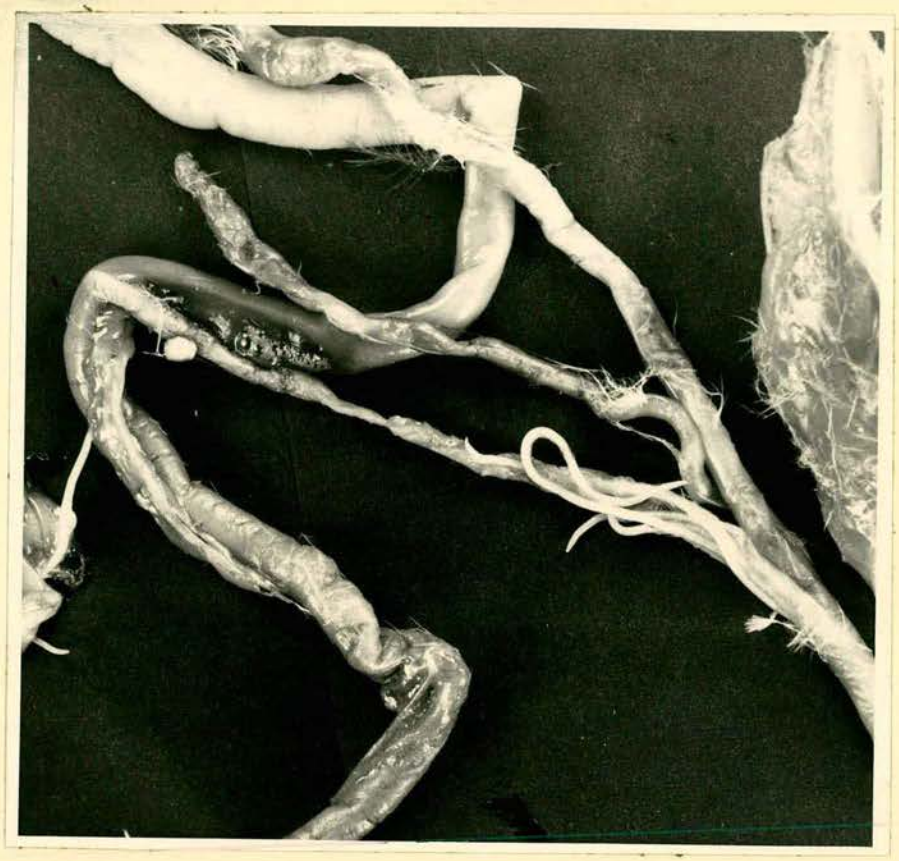


Plate 9: Hyperplasia of lymph nodules in infected (9a) and non-infected (9b) birds. (Experiment4).

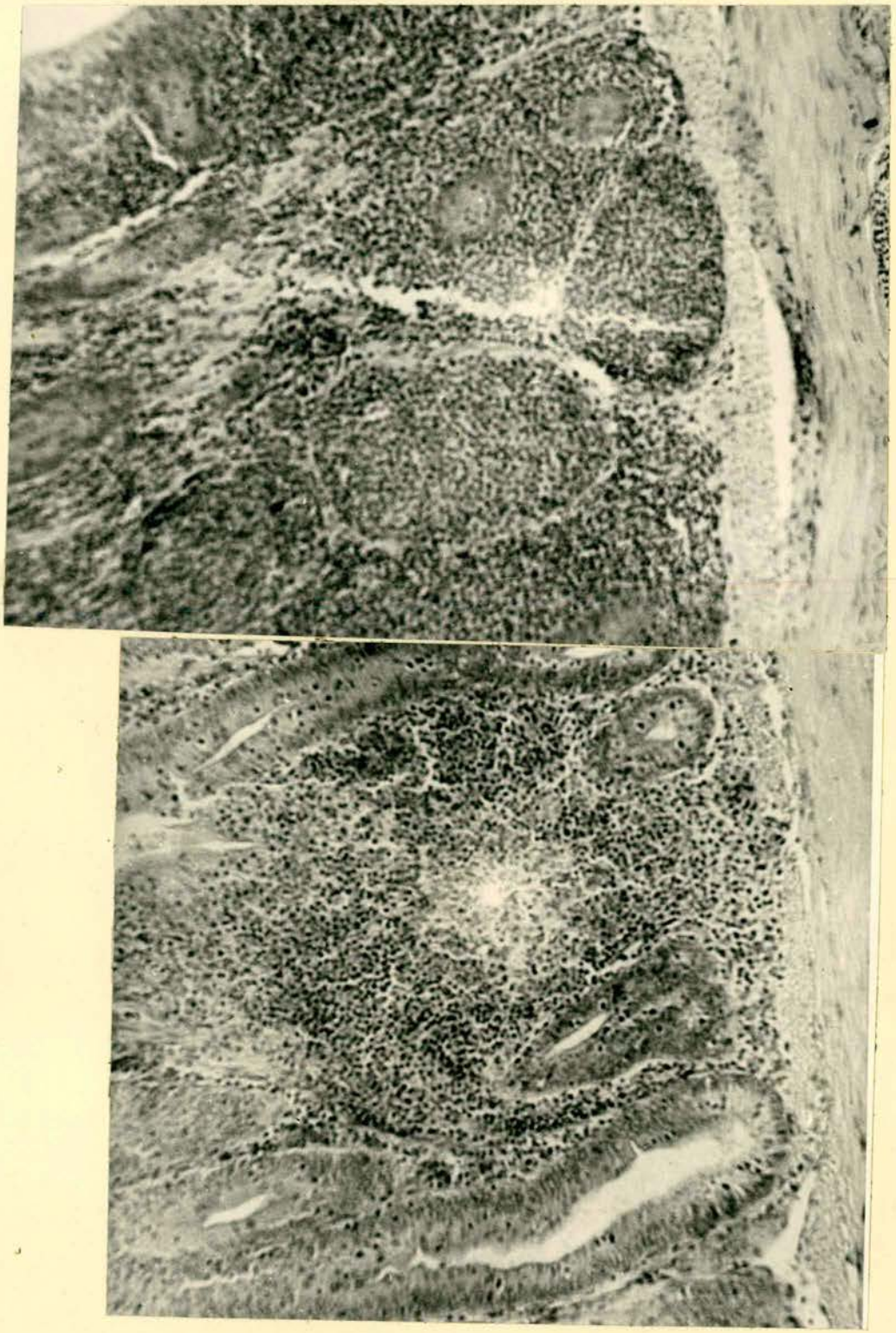


Plate 8: Dead larvae surrounded by fibroblasts. (Experiment 4).

Plate 7: Deep penetration of mucosa and bile duct (Experiment 3).

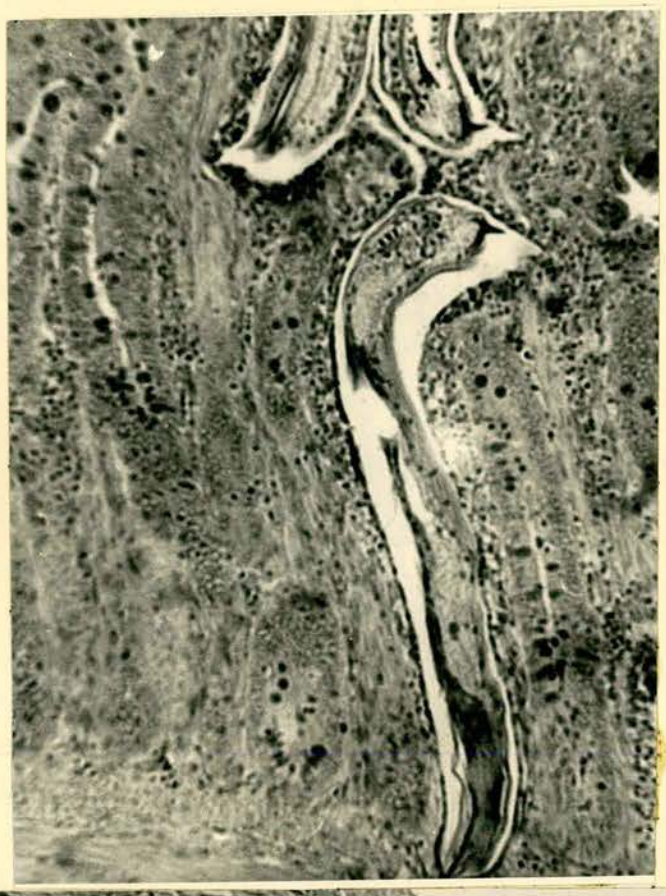


Plate 6: Duodenal mucous membrane in heavy infestation (Experiment 8).

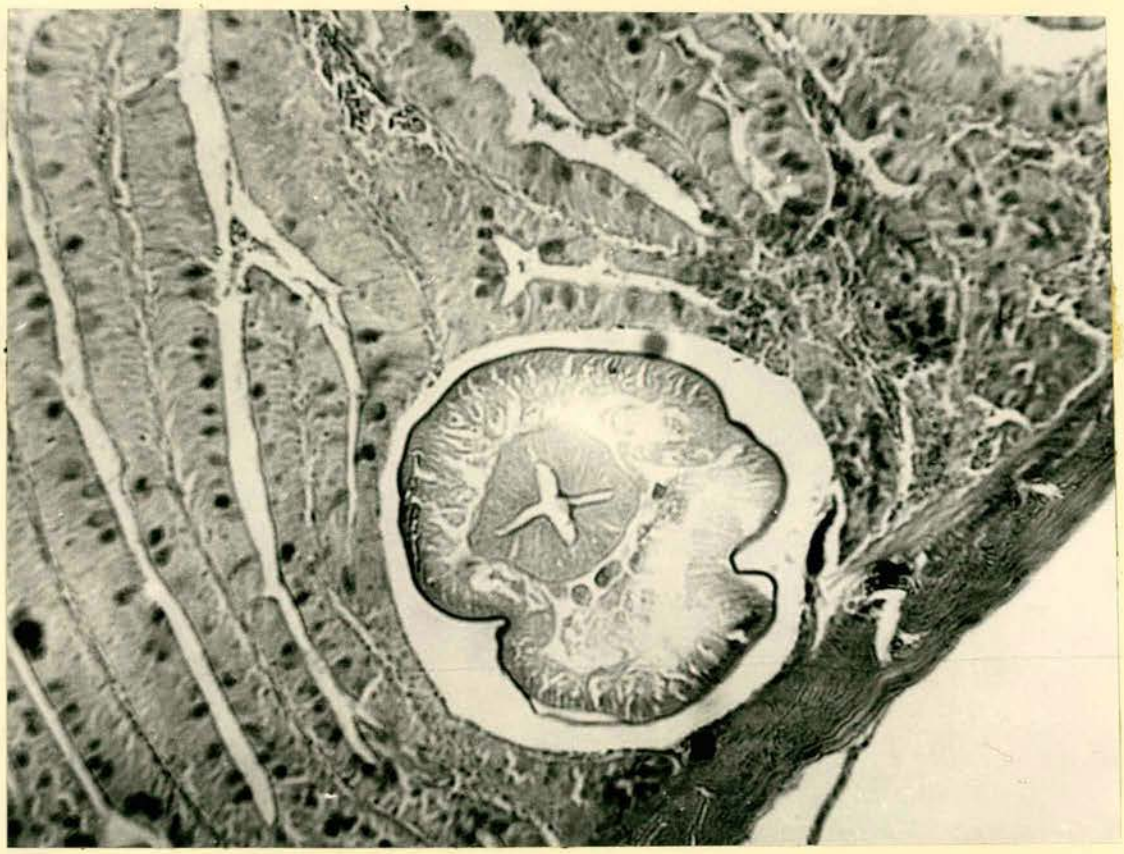
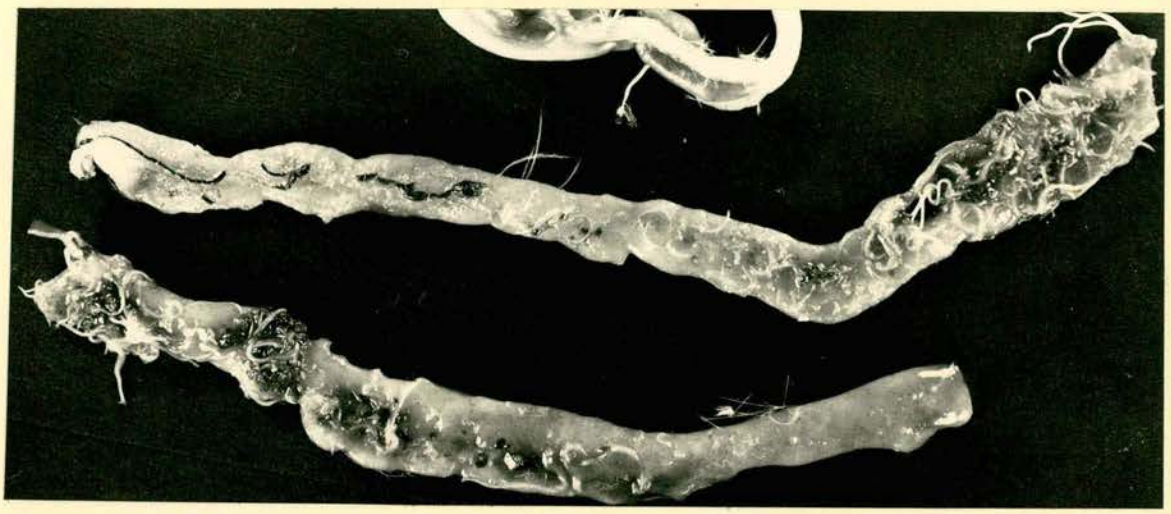


Plate 4: Duodenum of infected chicken (Expt. 3) showing severe haemorrhage into lumen.

Plate 5: Chicken with malnutrition and heavy infestation (Expt. 3) showing severe emaciation of breast muscles.

4



5.

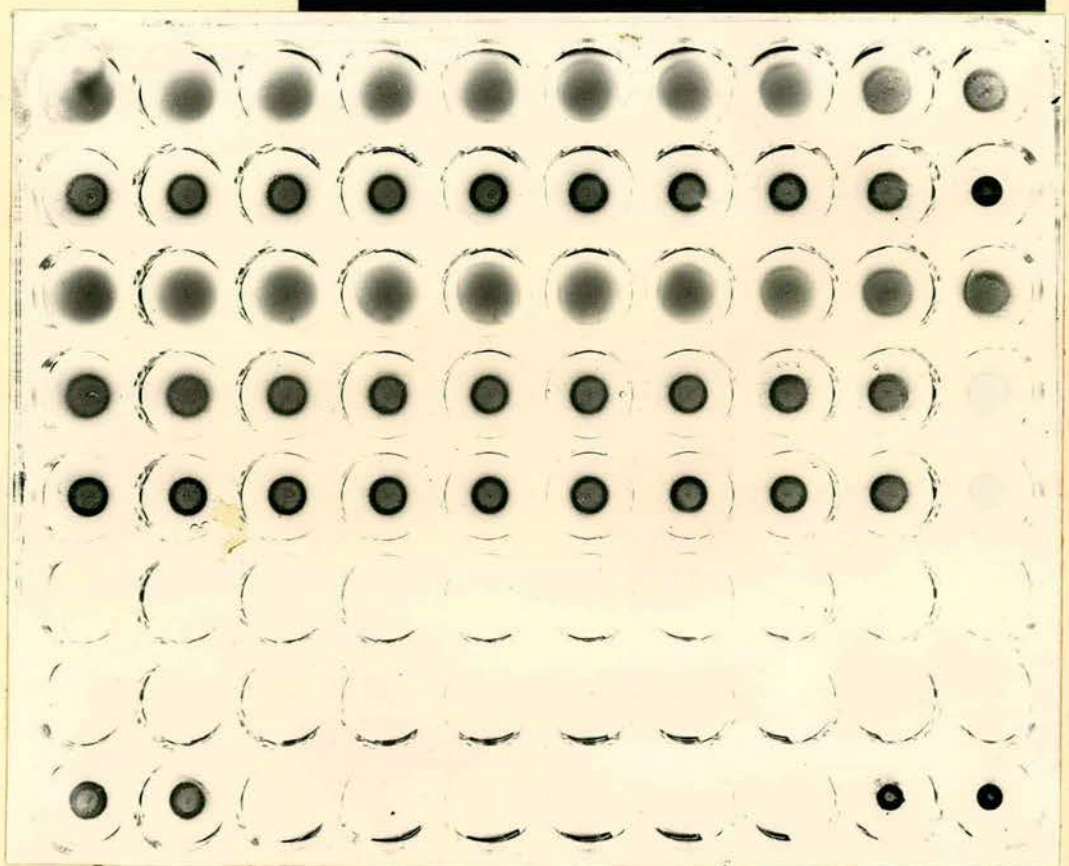
Plate 2: Ochtenlony plates: Cells An - Bovine serum albumin. As - Immune Poultry serum C- Normal poultry serum.

Plate 3: Haemagglutination test: chicken anti Bovine serum.

2.

3.

NORMAL CHICKEN SERUM



Buffered SALINE CONTROLS B. Saline controls